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MODERN MEDICAL HISTORY

THE RECOGNITION OF NEPHROGENIC DIABETES INSIPIDUS

A Very Small Page from the History of Medicine

The history of medicine is replete with the names of Hippocrates Galen and Avicenna, to say nothing of Lister and Semmelweiss. In its way this article also deals with medical history though it is concerned only with a very small piece of observation, bearing little significance for mankind if one takes the broad view It recounts in anecdotal form the recognition of two new genotypical cl nical entities both characterized by the symptom of diabetes insipidus The historical perspective is very short—little more than 30 years. In scientific matters anecdotal material is often regarded with some contempt My own view is a different one It seems to me that we can learn a good deal from anecdotes and strange coincidences may hold the seeds of scientific observation—seeds which deserve to be allowed to take root.

Howeve I would not have ventured to think of publishing the matter even as a small contribution to the history of medicine had not the Editor of this Journal requested me to do so He was present at a medical meeting and heard an informal talk of which this article is a part. The fact that this fragment appears in print is entirely the fault of Professor Jan Waldenström. It began in this way

In April 1940 the German armies occupied Swe den s neighbour country Norway *and a large number* of Swedish hospital doctors were called p for mili tary service Although not then fully qualified I was requested by the Chief of the Medical Department of the University Hospital Uppsala, Professor Gustaf Bergmark, to serve as relief house physician One of my first duties in this post concerned a man with diabetes insipidus A doctor in the provincial medical service—just as young as I and also acting as relief—telephoned to get some information from a hospital case paper He was attending a male pa tient E K who obviously had diabetes since there was polyuria and sugar was present in the urine On the other hand the urinary specific gravity was low and the output olume exceedingly high Did the patient have diabetes mellitus or diabetes insipidus The answer was that he had both—a lifelong sufferer from diabetes insipidus had acquired a mild diabetes mellitus of maturity type.

Study of the patient s case record revealed in the main the information about him and his siblings set out in Fig. 1 in which E K is no 9 in generation III He was one of 13 siblings—eight men and five women Diabetes insipidus had affected certainly four and possibly a fifth of the eight brothers but not one of the fi e sisters. In the evening I looked up the available genetic textbooks They stated that diabetes insipidus in its genetic form was inherited as an autosomal dominant character and the main reference quoted was the gigantic German genealogical tree which had been published by Adolph Weil (7) his son Alfred Weil (8) and the latter s son-in-law Camerer (1).

However a dominant autosomal transmission accorded badly with my set of siblings If four (or perhaps five) of eight brothers get the disease but not even one of five sisters this raises strong suspicion rather of a sex-linked transmission On the basis of this single set of siblings alone it was justifiable to conclude that here a dominant transmission carry ing equal risk for the two sexes was highly improbable

I searched old case record and visited a number of relatives of the 13 brothers and sisters An old brother of E K s mother born in 1854 (II.2) came to light and his diabetes insipidus was recorded in hospital case papers. Two sons of one of E K s sisters were also traced both had been treated in

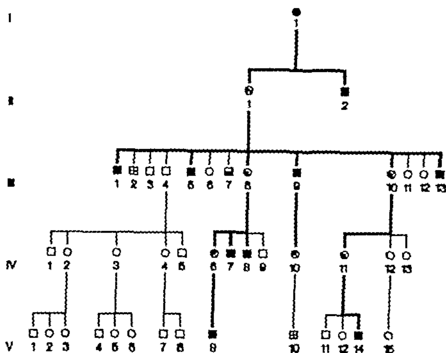


Fig 1 Pedigree representing nephrogenic diabetes insipidus, inherited as a recessive X-chromosome-linked trait. □=healthy male, ○=healthy female ■=affected male ⊞=possibly affected male, ⊙=conductor ◉=unknown individual in earlier generation presumably gene bearer ⊞⊕=dead in early infancy

hospital for diabetes insipidus. The sex-linked transmission became quite obvious

The secretary of the department where I was working noticed that I was searching for cases of familial diabetes insipidus She told me that her other had friends in Tierp a small town north of psala, and that in the family of these friends there s a series of persons with diabetes insipidus Was there then another stock and if so was it connected in any way with E K s family? There followed a visit to an old lady in Tierp She telephoned at once to her middle-aged son who himself suffered from diabetes insipidus though he had never been treated in hospital However there was his maternal grand father and sure enough I found that he figured in the case records of the University Hospital with this diagnosis The old lady had a family bible in which the names of a series of relatives were recorded. Mother and son sat with me pointed to various names and told me who had suffered from "the thirst" and who had not. It was exclusively men that they reported as affected and in this family the pattern of X-chromosome linkage was clear from the start. The family tree stretched away to Dalecarlia in central Sweden—there seemed to be no end to it. As the genealogical tangle unravelled the family became overwhelmingly large. Fig. 2 shows a single one of eight branches of a genealogical tree which four generations further back originated in a common woman ancestor born in 1712. However it also became clear from the genealogical study that no connection could be shown between the K siblings and the new dynasty from Tierp and Dalecarlia.

The line of clinical study picked its way forward in the following way In the thirties, amidopyrine had been used in the therapy of diabetes insipidus The rationale of this treatment was based on an observation by an Austrian D Scherf that amidopyrine blocks the effect of mercurial diuretics in the treat ment of oedema. Two patients with acquired diabetes insipidus who were under treatment in our Medical Department clearly received some benefit from amidopyrine therapy E. K and one of his brothers had been given amidopyrine but neither of them showed the least response to this substance However in the forties we had available various preparations of the antidiuretic hormone When E. K was 52 years old a trial was made of vasopressin pituitrin and tonephin but even with large doses not the least effect was discernible on urinary volume, chloride output or specific gravity A son of one of E. K s sisters willingly cooperated in a trial of the same preparations He was a youth of 17 years, mentally slightly backward but physically healthy In his case

Fig 2 Pedigree representing pituitary diabetes insipidus, inherited as recessive, X-chromosome-linked trait. Symbols as in Fig 1

there was no reason to consider impaired renal function due to age or any other cause Like his uncle E. K., he showed no vestige of effect from the antidiuretic hormone As biological proof of the effectiveness of the preparation we divided the contents of the ampoule and gave half to a healthy volunteer: he showed a powerful antidiuretic effect

At this time a woman with previously known diabetes insipidus had been admitted to the Maternity Department for the birth of her third child. Study of her family tree revealed dominant autosomal transmission that is the type described in the textbooks This form of the disease is undoubtedly much more common and I now know four such families in Sweden. Some kind members of this last family made the journey to undergo trial of the antidiuretic hormone All three who were investigated responded with a pronounced and typical antidiuretic effect.

The hypothesis now appeared to be temptingly simple: there was one form of diabetes insipidus inherited by autosomal dominant transmission, which responded to the antidiuretic hormone There was also another form, inherited as a sex chromosome-linked recessive which was refractory to the hormone

Study of the literature brought out the long-known fact that hereditary diabetes insipidus affects *men* more often than women. This had led to some rather far-fetched speculations about the pathophysiology of the disease. It was thought that testosterone played some part in the physical manifestation of the gene A German Lickint had written a paper entitled Ist der Diabetes Insipidus eine genito-hypophysäre Erkrankung?" Lickint gave the answer "yes" to his own question, his principal argument being the pronounced excess of males among those with the disease. His paper was highly speculative but it nevertheless did supply a stimulus. If the disease exists in different forms and if one of these forms is inherited by X-linked recessive transmission, then this could be the whole explanation of the excess of males found in the various case series. For example Hanhart (4) compiled a group of family trees with a total of 169 cases of the disease Of these 108 were males representing a significant deviation from the expected proportion 50 50

It was necessary to compile a series made up entirely of undoubted cases of autosomal transmission. *This could be done by including only those* from family trees which showed transmission of the disease from father to son, an impossibility with X linked inheritance I collected from the literature 13 pedigrees of this type: this produced 90 males and 63 females In any such compilation there is a selection factor involved which over weights the males, i.e. the requirement of inheritance from father to son. Those two members *in each family who are responsible for this selec* tion effect must be subtracted and when this correction is made the sex distribution becomes 64 males against 63 females. There was no longer any point in speculating about the effect of testo-

sterone on pathophysiology At all events the preponderance of males is fully explained by the existence of X-linked genes in certain families.

During this time new events had occurred on the clinical side It had to be confirmed that the X-linked recessive gene always implies a refractory state towards the antidiuretic hormone. A number of men from many parts of the country kindly agreed to be admitted to our Medical Department or to be investigated at their local hospitals for a trial of the hormone The majority belonged to the huge family tree which had its origin in Dalecarlia. The transmission was unquestionably X-chromosome-linked recessive Nine men of this lineage were tested with pituitrin All nine showed a prompt response thus refuting the hypothesis In other words the neatly streamlined explanation postulating two genetic patterns and two clinical forms was incorrect.

However from the original smaller family of E K and his 12 siblings four members could be tested with the hormone and all proved to be refractory It gradually became evident that this family represented a separate form of the malady which also had its own special character among other things a much more severe type of disease. Further study made it possible to define two different X-chromosome-linked genetic disorders with diabetes insipidus, which had not previously been distinguished Like the commoner autosomal and dominant inherited form of the disease e of these X linked forms responds to the antiuretic hormone. In fact no clinical difference can be demonstrated between these two distinct genetic entities with their different types of transmission. The other X-linked form of the disease is what later came to be termed renal or nephrogenic diabetes insipidus. It is refractory to the hormone and so must have a completely different pathophysiology

Among the special risks of the nephrogenic form of the disease must be included the risk of mental retardation This association became clear to me only after my thesis on diabetes insipidus (3) was already in press However quite early on one mother had told the following story At a very early age her first son was remarkably thirsty The family doctor advised restriction of the child s naughty drinking The boy had constant fever and his bowel function was extremely sluggish His development ceased he became emaciated and he died at the age of 18 months Seven years later this woman had her second son and everything began in the same way She was again advised to restrict fluids but this time she disobeyed the instructions and gave the boy as much water as he wanted He is still alive healthy and able to work but he is backward and had to attend an ESN school This account of the two brothers perhaps contains a good deal of suggestion but as will appear later the suggestion is not without justification

During Christmas 1943 I received a visit from an old friend the paediatrician Edgar Mannheimer who later died during his service as professor and head of the Ethiopian Swedish Paediatric Clinic in Addis Ababa. We were talking about diabetes insipidus and Mannheimer said that in his children s hospital in Stockholm he had recently seen an extraordinary boy aged two years The child had protracted fever without any signs of infection for months he had had a swinging temperature often very high His growth and development had stagnated and he had chronic constipation, with faeces resembling mortar Gradually it was noted that the child was avid for water and could consume astonishing quantities The diagnosis of diabetes insipidus had ultimately been made but only after long delay When he finally got a liberal supply of water his alarming condition subsided and he began to flourish Mannheimer happened to mention the boy s surname which is a very uncommon one. It made me start, for this name was very familiar to me from E K s family There was no difficulty in establishing that the boy was a son of the daughter of one of E. K s sisters (V 9 in Fig 1)

The head of the Children s Clinic Crown Princess Lovisa s Hospital in Stockholm was Professor Adolf Lichtenstein I was able to show him a case paper from the University Hospital in Uppsala written in 1905 by the recently qualified house physician Adolf Lichtenstein and referring to a patient who was a brother of the maternal grandmother of the boy then under treatment in the Crown Princess Lovisa s Hospital (III 5). The case paper had been written with great care and it recorded a history taken from relatives which described the patient s swinging fever emaciation and severe constipation at an early age. The descendant at that time under Lichtenstein s care recovered well and was discharged a few months after he began to receive liberal amounts of water However he remained mentally backward and received his schooling in an institution for the mentally retarded.

Yet another coincidence taught me to recognize he paediatric picture of nephrogenic diabetes insipidus. In the winter of 1944–45 while reading the proofs of my thesis I was called up for the civil defence service at a place in the far north of Sweden We had to care for those of the civil population who had been removed from the evacuated areas during the final hostilities between Finland and Russia, mainly mothers and children. I was responsible for an emergency hospital and for the paediatric work I had the help of the chief of a nearby children s hospital One evening this colleague told me that he was worried about a most unusual case in his hospital a 1 year-old boy who seemed likely to die. He had high swinging fever without any other signs of infection wasting and severe constipation. That boy has diabetes insipidus I declared and I got the ery reasonable reply that my proof reading had gone to the brain and that there really were some other kinds of disease too! We hurried to the children s hospital The clinical history was strikingly similar to that of the boy at Professor Lichtenstein s hospital in Stockholm two years earlier I asked if I might telephone the mother of the child and my colleague agreed though he thought it rather odd I asked the mother whether she had any relatives with the unusual name of the previous boy The answer was "yes" she had cousins of that name down in the Uppsala–Stockholm region and she herself was born just outside Uppsala. Lichtenstein s patient and this boy (V 14 Fig. 1) were second cousins, their mothers were first cousins and their maternal grandmothers were both sisters of E. K.

We returned to the boy and gave him liberal drinks of water Next day he was afebrile and remained so H began to gain weight and about a month later he was discharged from the hospital in good health However he was found to be mentally retarded and later attended school in an institution for the mentally deficient

Nephrogenic diabetes insipidus is nowadays included in the textbooks as a cause of mental retardation It is also clear that if the diagnosis is not recognized in time there is risk of early death In couple of later publications I put forward the view that cerebral damage results from early dehydration, either d rectly or through some t xic effect The litre of milk which forms the routine feed of me for young nfants cannot replace these infant w ter los If the mother is known heterozygote all her male children should be plied with water until they reach an age at which the diagnosis can be confirmed or excluded.

This sketch of the way in which the observations on the two different forms of X-chromosome-linked hereditary diabetes insipidus meandered their way forward through a series of coincidences is not recalled mainly from memory I still have detailed notes and photo-copies of the original case records.

In 1945 that is in the same year as my thesis appeared Waring, Kajdi and Tappan (6) published quite independently of me their observation of nephrogenic diabetes insipidus in six boys of whom three were half-brothers. They described the clinical picture in early infancy and pointed out that the antidiuretic hormone i without effect.

This then was one of those coincidences of which one is wont to use the expression "the time was ripe It is clear that the observation was made independently in the USA and in Sweden I published it in a preliminary form in 194 (7) and therefore had no knowledge of the paper by Waring and his colleagues of 1945 My paper of 1942 was published in a Swedish language jour nal although with a short summary in English. It is not reasonable to suppose that the American workers could have been acquainted with it.

At the time of their discovery many new disease entities appear to be extremely rare The fact that all my patients could soon be shown to belong to the same family also gave this impression Later it became clear—and this is how things usually are—that nephrogenic diabetes insipidus is not so extraordinarily rare I have been n contact with two other families in Sweden who are affected by this disease and it is known in many other parts of the world.

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NUCLEOLAR SIZE IN BENIGN AND MALIGNANT PLASMA CELL PROLIFERATION

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Abstract Nucleolar and nuclear size of bone marrow plasma cells has been studied in 26 cases of myeloma, 19 of benign essential monoclonal gammapathy (BEMG) and 9 without an M-component. Bone marrow smears were Feulgen-stained to visualize nucleoli and nuclei. Nucleolar area, nuclear area and the ratio nucleolar area/nuclear area were calculated. The myeloma group differed from the other tw groups in having plasma cells with larger nucleoli, larger nuclei and an increased ratio nucleolar area/nuclear area. No difference was found between the BEMG group and the cases without an M-component. In some cases—in which the absence of osteolytic lesions at X-ray examination, low plasma cell percentage in bone marrow smears or low M-component concentration initially made the diagnosis of myeloma uncertain—the observation of enlarged nucleoli could help to establish the diagnosis. Three cases were observed in which the onset of myeloma was preceded by long period of essentially unchanged M-component concentration. In these cases sudden increase of M-component concentration was accompanied by an increase of mean nucleolar size of bone marrow plasma cells.

Multiple myeloma is a disease characterized by a monoclonal proliferation of immunoglobulin-producing cells in the bone marrow Their product can be demonstrated as an M-component in serum and/or Bence Jones protein in urine M-components also occur however in persons without clinical cytological, or radiological signs of multiple myeloma, as was first pointed out by Waldenström (16) The term benign essential monoclonal gammapathy (BEMG) has later been applied to this condition (18) While multiple myeloma is a malignant tumor which, if untreated grows until the death of its host BEMG can be regarded as a benign tumor which has not quite escaped the homeostatic control of proliferation. At a certain size the growth of the tumor is slowed down so that steady state is achieved. BEMG occurs in 1% of the population over 25 years of age and in about 3% of people over 70 years. Its incidence is about 40 times that of myeloma (2) When an M-component is discovered it is thus essential to decide whether the underlying condition is a myeloma, a macroglobulinemia Waldenström or a BEMG This is all the *more important as we have now a treatment for* myeloma and macroglobulinemia which is effective but far from being without danger

The differential diagnosis of multiple myeloma and BEMG can be difficult. In early stages of multiple myeloma osteolytic bone lesions can often not be demonstrated by X-ray In general myeloma differs from BEMG in having more plasma cells in bone marrow smears, higher concentration of M component in serum, and larger quantities of monoclonal light chains in urine There is however a wide overlap between the two conditions in these respects (9) In many cases the only way to decide whether myeloma or BEMG is present is continued observation of the patient. An unchanged concentration of the M-component for several years is a strong indication of BEMG (9 17). The aim of this investigation was to find out whether morphological differences between the plasma cells in BEMG and myeloma can facilitate the establishment of the differential diagnosis of these two conditions

MATERIAL

The material consists of 54 patients, 26 with myeloma, 19 with BEMG and 9 without an M-component (Table I) Three cases of myeloma with long static phase before the *outbreak of signs of typical myeloma are reported separately* All cases have been examined in the Department of

Table I *Description of the material*

No M-component	9
a Polyclonal Ig increase	4
b Normal Ig concentration	5
BEGM	19
a M-component type IgG	13
b M-component type IgA	5
c M-component type IgM	1
Myeloma	26
a M-component type IgG	17
b M-component type IgA	6
c Bence Jones protein	3
BEGM developing into myeloma type IgG	3

Medicine Malmö General Hospital during 1965–71 Four of the cases without an M-component had a polyclonal immunoglobulin increase, while five had normal immunoglobulin concentrations. Eight BEMO cases were selected because of plasma cell percentages above 5% in bone marrow smears. All other cases were unselected

The diagnostic criteria for multiple myeloma were as follows. 1) M-component in serum and/or Bence Jones protein in urine 2) Osteolytic bone lesion demonstrated by X-ray (not only osteoporosis) without any other apparent explanation than a plasma cell proliferation (more than 3% plasma cells in bone marrow smears) or macroscopic lytic bone lesions demonstrated at autopsy corresponding to a proliferation of plasma cells.

Cases fulfilling the following criteria were classified as BEMO 1) M-component in serum 2) No osteolytic bone lesions at X-ray examination and/or no macroscopic lytic bone lesions at autopsy 3) Unchanged M-component concentration or an increase at repeated examination of not more than 0 5 mg/100 ml during an observation period of at least three years

Most cases were under observation for far more than three years. When the study was interrupted, 13 patients were dead All but one of these were autopsied Two cases who showed no signs of myeloma at autopsy were accepted as BEMO even though the observation period was slightly shorter than three years. Six patients were still alive at the end of the study The average observation period for all BEMO cases was seven years In two cases the M-component disappeared during the observation period. In all myeloma cases except five bone marrow examination was done before cytostatic therapy was started. In the BEMO cases bone marrow examination was done when the M-component was discovered in some and later during the observation period in others

METHODS

Bone marrow was aspirated by sternal puncture and smears were made by conventional slide technique Slides were stained with May-Grünwald-Giemsa and 1 000 cells were counted to calculate the plasma cell percentage To demonstrate nucleoli, slides were Feulgen-stained after fixation in absolute methanol for 10 min (6). Contrast staining of the cytoplasm with light green was used to



Fig 1 Bone marrow plasma cells in a case of BEMO (*a*) and a case of myeloma (*b*) Feulgen light green. ×1600.

facilitate the identification of plasma cells. With this technique it was possible to demonstrate in the majority of the plasma cells a single round or oval, eccentrically placed nucleolus, separated from surrounding nuclear DNA by a membrane-like structure of condensed chromatin (Fig 1) Intranuclear inclusions occurred in some myeloma cells but were as a rule easily distinguished from nucleoli. With a Zeiss ocular micrometer two diameters of nucleolus and of nucleus were measured, and the areas were calculated using the formula for the area of an ellipse If two or more nucleoli occurred in the same cell the areas were lumped together In each slide 25–30 cells were measured and the means of nucleolar area, nuclear area and relative nucleolar area (nucleolar area/nuclear area ×100) were calculated By measuring 25 cells S.E M of less than 5% was obtained. In varying number of plasma cells (10–20%) the demarcation of the nucleolus from the nucleus was not quite sharp and these cells were omitted There was no correlation between the number of such cell and mean nucleolar or nuclear size

Protein studies were made in the Department of Clinical Chemistry M-component concentrations were estimated in most cases by paper electrophoresis and protein determination after separate elution of the M-component (3

Table II. *Nucleolar area nuclear area relative nucleola area and plasma cell percent ge i 9 patients without an M-component*

Pat. no	Diagnosis	Plasma cell per-centage	Nucelolar area (μ) Mean	Nucelolar area (μ) S.D	Nuclear area (μ^2) Mean	Nuclear area (μ^2) S.D	Relative nucleolar area Mean	Relative nucleolar area S D
1	Rheum arthritis	2.0	5.3	1 1	51.3	9.3	10 4	2
2	Monocytic leukemia	2.7	5.8	2.0	60 2	11	9.6	3 0
3	Collagenosis	1 1	5.2	1 1	59 4	12.5	8.8	1 3
4	Rheum. arthritis	2.1	5 8	1 9	53.8	11 5	11 0	3 1
5	F lty s syndrome	1 9	6 3	1.9	59 0	10 7	10 7	7
6	Condyloma acum.	.6	5 7	1 4	63 0	11 3	9 1	2.1
7	Myocardial inf	2.7	4.2	1 1	50.3	10 4	8.4	1.8
8	Renal cancer	2.9	5 9	1.2	61.5	13 3	9 9	1 9
9	Cancer of uterine cervi.	1.0	5 1	1.3	62.9	12.3	8 2	1.6
Mean		2.1	5.5		57 9		9 6	
S.D		0.7	0 6		4 9		1 0	

9). In some cases electroimmunoassay according to Laurell (10) was used. Classification of M-components was made using immunoelectrophoresis w th monospecific antisera.

RESULTS

The values for mean nucleolar area nuclear area relative nucleolar area, and plasma cell percentage in 9 cases without an M-component are given in Table II If the measured values from all cases are pooled (370 cells) typical normal variation curves are obtained for nucleolar as well as nuclear area. The number of measured cells in each case is too small to allow a definite statement of normal variation. However no tendency to a bimodal variation was observed Tables III and IV show the corresponding values for the BEMG and the myeloma cases

Table III. *Nucleolar area uclear area relative nucleolar area and plasma ell percentage in 19 BEMG patients*

G=light chain type not determined

Pat. no	M-component Type	M-component Conc. (g/100 ml)	Plasma cell per centage	Nucleolar area (μ^2) Mean	Nucleolar area (μ^2) S.D	Nuclear area (μ^2) Mean	Nuclear area (μ^2) S D	Relative nucleolar area Mean	Relative nucleolar area S D
1	GK	1.6	3 9	7 2	3 1	62.4	7 0	12.2	4.2
	GK	.0	3 7	5 0	1.5	48 5	9 7	10 4	1 9
3	GK	0 8	6.1	4.5	1 0	58 6	12.0	7 9	1 8
4	GK	2.8	11.0	5.2	1.5	65 9	14.0	8.0	1 7
5	GK	1.2	9.5	7.2	2 6	56 8	10 6	L 5	3 2
6	GK	1 7	6.6	4 9	1 1	80 0	9 4	6 2	1 2
7	GK	1.	0.7	3 7	0 9	52.0	9 1	7 3	1.3
8	GL	1 7	3 0	6 8	.2	57 7	21.5	11 3	1 4
9	GL	1.3	3 7	6.5	1 4	59 5	10 2	11 0	2.2
10	GL	1 1	3.3	7.2	2.4	6 1	10 4	11 5	9
11	G	2.0	5 4	4.5	1 0	48 2	8 8	9 4	2 1
12	G	1.2	4 6	4 2	0 9	50 6	10 9	8 4	1 7
13	G	1.2	3.3	3 6	1.0	37 5	5 9	9 7	2 3
14	AK	0 8	2.6	10 2	6	93 1	18 2	10 8	1 6
15	AK	0 6	3 4	7 5	1 6	58.2	9 4	13 0	2 2
16	AK	0.8	2 3	4 8	1 7	46 0	12 6	10 2	2 5
17	AK	1 6	1 8	5.3	1.8	66 6	14.8	8 3	2.6
18	AK	0.6	1 5	4 8	1.9	55.5	13 4	8 7	1 9
19	MK	0 9	5 6	5 3	1	51 7	10.1	10 5	2 3
Mean		1.3	4 3	5 7		58 5		9 9	
S.D		0 6	2 6	1 6		1 4		1 9	

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Abstract Nucleolar and nuclear size of bone marrow plasma cells has been studied in 26 cases of myeloma, 19 of benign essential monoclonal gammapathy (BEMG) and 9 without an M-component. Bone marrow smears were Feulgen-stained to visualize nucleoli and nuclei. Nucleolar area, nuclear area and the ratio nucleolar area/nuclear area were calculated. The myeloma group differed from the other two groups in having plasma cells with larger nucleoli, larger nuclei and an increased ratio nucleolar area/nuclear area. No difference was found between the BEMG group and the cases without an M-component. In some cases—in which the absence of osteolytic lesions at X-ray examination, low plasma cell percentage in bone marrow smears or a low M-component concentration initially made the diagnosis of myeloma uncertain—the observation of enlarged nucleoli could help to establish the diagnosis. Three cases were observed in which the onset of myeloma was preceded by long period of essentially unchanged M-component concentration. In these cases a sudden increase of M-component concentration was accompanied by an increase of mean nucleolar size of bone marrow plasma cells.

Multiple myeloma is a disease characterized by a monoclonal proliferation of immunoglobulin-producing cells in the bone marrow Their product can be demonstrated as an M-component in serum and/or Bence Jones protein in urine M-components also occur however in persons without clinical cytological or radiological signs of multiple myeloma, as was first pointed out by Waldenström (16). The term benign essential monoclonal gammapathy (BEMG) has later been applied to this condition (18) While multiple myeloma is a malignant tumor which, if untreated grows until the death of its host, BEMG can be regarded as a benign tumor which has not quite escaped the homeostatic control of proliferation. At a certain size the growth of the tumor is slowed down so that a steady state is achieved. BEMG occurs in 1 % of the population over 25 years of age and in about 3 % of people over 70 years Its incidence is about 40 times that of myeloma (2) When an M-component is discovered it is thus essential to decide whether the underlying condition is a myeloma, a macroglobulinemia Waldenström or a BEMG This is all the more important as we have now a treatment for myeloma and macroglobulinemia which is effective but far from being without danger

The differential diagnosis of multiple myeloma and BEMG can be difficult. In early stages of multiple myeloma osteolytic bone lesions can often not be demonstrated by X-ray In general myeloma differs from BEMG in having more plasma cells in bone marrow smears higher concentration of M component in serum and larger quantities of monoclonal light chains in urine There is however a wide overlap between the two conditions in these respects (9) In many cases the only way to decide whether myeloma or BEMG is present is continued observation of the patient. An unchanged concentration of the M-component for several years is a strong indication of BEMG (9 17) The aim of this investigation was to find out whether morphological differences between the plasma cells in BEMG and myeloma can facilitate the establishment of the differential diagnosis of these two conditions

MATERIAL

The material consists of 54 patients, 26 with myeloma, 19 with BEMG and 9 without an M-component (Table I). Three cases of myeloma with long static phase before the outbreak of signs of typical myeloma are reported separately All cases have been examined in the Department of

Table I *Description of the material*

No M-component	9
a Polyclonal Ig increase	4
b Normal Ig concentration	5
BEGM	19
a M-component type IgG	13
b M-component type IgA	5
c M-component type IgM	1
Myeloma	26
a M-component type IgG	17
b M-component type IgA	6
c Bence Jones protein	3
BEGM developing into myeloma type IgG	3



Fig 1 Bone marrow plasma cells in a case of BEMG (*a*) and case of myeloma (*b*). Feulgen light green. ×1600.

Medicine Malmö General Hospital, during 1965–71 Four of the cases without an M-component had a polyclonal immunoglobulin increase while five had normal immunoglobulin concentrations. Eight BEMG cases were selected because of plasma cell percentages above 5% in bone marrow smears. All other cases were unselected

The diagnostic criteria for multiple myeloma were as follows: 1) M-component in serum and/or Bence Jones protein in urine.) Osteolytic bone lesions demonstrated by X-ray (not only osteoporosis) without any other apparent explanation than plasma cell proliferation (more than 3% plasma cells in bone marrow smears) or macroscopic lytic bone lesions demonstrated at autopsy corresponding to proliferation of plasma cells.

Cases fulfilling the following criteria were classified as BEMG 1) M-component in serum. 2) No osteolytic bone lesions at X-ray examination and/or no macroscopic lytic ne lesions at autopsy 3) Unchanged M-component con- or an increase at repeated examination of not than 0.5 mg/100 ml during an observation period of least three years.

Most cases were under observatio for far more than hree years When the study was interrupted, 13 patients ere dead. All but one of these were autopsied Two cases, who showed no signs of myeloma at autopsy were accepted as BEMG even though the observation period was slightly shorter than three years Six patients were still alive at the end of the study The average observation period for all BEMG cases was seven years In two cases the M-component disappeared during the observation period. In all myeloma cases except five bone marrow examination was done before cytostatic therapy was started. In the BEMG cases bone marrow examination was done when the M-component was discovered in some and later during the observation period in others

METHODS

Bone marrow was aspirated by sternal puncture and smears were made by conventional slide technique. Slides were stained with May-Grünwald-Giemsa and 1000 cells were counted to calculate the plasma cell percentage To demonstrate nucleoli, slides were Feulgen-stained after fixation in absolute methanol for 10 min (6) Contrast staining of the cytoplasm with light green was sed to facilitat the identification of plasma cells With this technique it was possible to demonstrate in the majority of the plasma cells a single, round or oval, eccentrically placed nucleolus, separated from surrounding nuclear DNA by membrane-like structure of condensed chromatin (Fig 1). Intranuclear inclusions occurred in some myeloma cells but were, as rule easily distinguished from nucleoli With Zeiss ocular micrometer two diameters of nucleolus and of nucleus were measured and the areas were calculated sing the formula for the area of an ellipse If two or more nucleoli occurred the same cell the areas were lumped together In each slide 25–30 cells were measured and the means of nucleolar area, nuclear area and relative nucleolar area (nucleolar area/nuclear area ×100) were calculated. By measuring 25 cells S.E M. of less than 5% was obtained In varying number of plasma cells (10–20%) the demarcation of the nucleolus from the ucleus was not quite harp and these cell were omitted There was no correlation between the number of such cells and mean nucleolar or nuclear size.

Protei studies were made in the Department of Clinical Chemistry M-component concentrations were estimated in most cases by paper electrophoresis and protein determination after separate lution of the M-component O

Table II *Nucleolar area nuclear rea elative nucl olar area and plasma cell percentage in 9 patients without an M-component*

Pat. no	Diagnosis	Plasma cell pe centage	Nucleolar area (μ^2)		Nuclear area (μ^2)		Relative nucleolar area	
			Mean	S D	Mean	S D	Mean	S D
1	Rheum. arthritis	2 0	5.3	1 1	51.3	9.3	10 4	2
2	Monocytic leukemia	..7	5.8	2.0	60.2	11 2	9 6	3 0
3	Collagenosis	1 1	5..	1 1	59 4	12.5	8.8	1 3
4	Rheum. arthritis	..1	5 8	1 9	53.8	11.5	11.0	3 1
5	*Felty syndrome*	*1 9*	6.3	*1 9*	*59 0*	*10 7*	*10.7*	2.7
6	Condyloma acum.	6	5 7	1 4	63.0	11 3	9 1	1
7	Myocardial inf	2.7	4.2	1 1	50 3	10 4	8 4	1 8
8	Renal cancer	2.9	5 9	1	61.5	13 3	9 9	1 9
9	Cancer of uterine cervix	1.0	5 1	1.3	62 9	12 3	8 2	1 6
Mean		2.1	5.5		57 9		9 6	
S.D		0 7	0.6		4.9		1 0	

9). In some cases electroimmunoassay according to Laurell (10) was used. Classification of M-components was made using immunoelectrophoresis with monospecific antisera.

RESULTS

The values for mean nucleolar area, nuclear area relative nucleolar area and plasma cell percentage in 9 cases without an M-component are given in Table II If the measured values from all cases are pooled (370 cells) typical normal variation curves are obtained for nucleolar as well as nuclear area. The number of measured cells in each case is too small to allow a definite statement of normal variation How ever no tendency to a bimodal variation was observed Tables III and IV show the correspond ng values for the BEMG and the myeloma cases

Table III. *Nucleolar area nuclear area relative nucleola are and plasma cell perc entage in 19 BEMG patients*

G = light chain type not determined

Pat. no	M-component		Plasma cell per centage	Nucleolar area (μ^2)		Nuclear area (μ^2)		Relative nucleolar area	
	Type	Conc. (g/100 ml)		Mean	S.D	Mean	S.D.	Mean	S D
1	GK	1.6	3 9	7.2	3 1	62.4	7 0	1 2	4.2
2	GK	2 0	3 7	5 0	1.5	48 5	9 7	10 4	1 9
3	GK	0.8	6.1	4.5	1 0	58 6	12.0	7 9	1.8
4	GK	2.8	11 0	5.2	1 5	65 9	14.0	8 0	1 7
5	GK	1.2	9.5	7 2	2 6	56 8	10 6	12.5	3 2
6	GK	1 7	6 6	4 9	1 1	80 0	9 4	6..	1 2
7	GK	1.2	0.7	3 7	0 9	52 0	9 1	7 3	1.3
8	GL	1 7	3 0	6 8	2 2	57 7	21 5	11.3	1 4
9	GL	1 3	3 7	6 5	1 4	59.5	10 2	11.0	2 2
10	GL	1 1	3 3	7.2	4	62 1	10 4	11 5	9
11	G	2.0	5 4	4 5	1 0	48 2	8 8	9 4	2 1
12	G	1.2	4.6	4	0 9	50.6	10 9	8 4	1 7
13	G	1.2	3 3	3 6	1 0	37 5	5 9	9 7	2.3
14	AK	0.8	2.6	10 2	2 6	93 1	18	10 8	1 6
15	AK	0 6	3 4	7 5	1 6	58 2	9 4	13.0	..2
16	AK	0 8	3	4 8	1 7	46 0	12.6	10 2	2 5
17	AK	1 6	1 8	5 3	1 8	66.6	14 8	8 3	2 6
18	AK	0.6	1 5	4 8	1 9	55 5	13 4	8 7	1.9
19	MK	0.9	5 6	5 3	1 2	51 7	10.1	10 5	2.3
Mean		1.3	4 3	5 7		58 5		9 9	
S.D.		0.6	2 6	1 6		12 4		1 9	

Table IV *Nucleolar area, nuclear area, relative nucleolar area and plasma cell percentage in 26 myeloma patients*

BJ = Bence Jones proteinuria, G = light chain type not determined, n.d. = not determined

Pat. no.	M-component	Conc. (g/100 ml)	BJ	Plasma cell percentage	Nucleolar area (μ^2) Mean	Nucleolar area (μ^2) S.D.	Nuclear area (μ^2) Mean	Nuclear area (μ^2) S.D.	Relative nucleolar area Mean	Relative nucleolar area S.D.
1	GK	4.5	Trace	40	14.0	2.3	84.5	9.0	16.7	2.9
2	GK	1.6	Pos.	22	12.3	3.1	79.3	14.2	15.8	3.1
3	GK	4.0	Neg.	13	14.6	3.4	84.8	12.0	17.3	3.8
4	GK	1	Trace	3	12.8	4.3	89.6	18.3	14.2	4.3
5	GK	0.4	Neg.	2	7.1	3.4	73.1	15.3	9.6	3.9
6	GK	1.5	n.d.	10	17.8	7.1	94.3	28.9	19.0	5.8
7	GK	4.0	Pos.	27	5.9	1.4	69.9	10.9	8.5	1.8
8	GK	1.9	Trace	8	10.3	3.4	81.2	15.3	13.5	3.4
9	GK	2.3	Neg.	20	15.1	3.1	96.1	14.3	15.9	3.6
10	GK	7.0	n.d.	11	17.3	4.4	90.9	22.8	19.5	4.6
11	GK	3.2	Pos.	9	18.1	7.9	104.4	50.8	16.4	3.6
12	GL	4.8	Pos.	22	9.1	1.9	70.5	17.9	13.1	2.2
13	GL	2.6	Trace	3	9.7	2.7	84.6	17.7	11.7	2.6
14	GL	6.4	Pos.	25	10.2	3.2	69.6	18.3	14.7	3.3
15	GL	6.3	Pos.	5	11.8	3.8	55.6	7.4	21.3	4.2
16	G	3.4	Pos.	8	11.2	4.5	83.5	28.5	13.6	4.3
17	G	4.7	n.d.	8	8.0	1.4	66.5	13.0	12.3	2.0
18	AK	4.0	Neg.	28	14.1	3.1	80.9	15.6	17.6	2.9
19	AK	3.2	Pos.	25	12.0	4.3	75.1	12.5	15.7	1.9
20	AK	4.0	Neg.	12	11.4	4.3	85.1	25.3	13.8	1.6
21	AK	2.4	Neg.	10	14.1	4.0	96.1	19.1	14.6	3.7
22	AK	2.1	Trace	9	12.2	3.7	83.8	17.4	14.6	2.0
23	AL	1.2	Pos.	7	21.1	6.9	100.7	19.1	20.6	5.1
24	K	–	Pos.	12	13.4	3.8	78.5	14.1	17.0	3.4
25	L	–	Pos.	45	12.5	3.7	60.6	19.4	11	3.2
26	L	–	Pos.	30	11.4	3.9	72.9	17.0	15.8	4.9
Mean		3.4		15.9	12.6		81.[illegible]		15.5	
S.D.		1.7		11.5	3.5		1[illegible].0		3.2	

Figs. 2, 3 and 4 demonstrate nucleolar area, nuclear area and relative nucleolar area for the three groups of patients. Each point represents the mean of 25–50 measured cells in one slide. In three cases of myeloma with a long benign prephase the cells were measured at the stage when the M-component was discovered (the condition was then judged as BEMG) and in the first bone marrow aspirate made after a sudden increase in the M-component concentration was registered (the condition was then classified as myeloma).

It is evident that the myeloma group differs from the other groups in having plasma cells with larger nucleoli and larger nuclei. The ratio nucleolar area/nuclear area is also increased. The differences are statistically significant ($p<0.001$) for these three variables. The best separation of the myeloma group is obtained using nucleolar area. On the other hand there is no difference between the group without an M-component and the BEMG group.

The plasma cell percentages in bone marrow smears of the three diagnostic groups are demonstrated in Fig. 5. Again there is good separation between groups but overlapping is more prominent. In the three cases with a long benign stage, cell measurements were made several times during the course and the results are given in Fig. 6.

DISCUSSION

It is a well known fact that myeloma cells can differ from normal plasma cells in several respects: one or more large nucleoli, large nuclei of varying form, increased nucleo-cytoplasmic ratio, occurrence of binucleated cells, loose chromatin structure in the nuclei, etc. (9, 17). Myeloma cases with predominantly normal "mature" plasma cells are, however, not uncommon. Fadem (7) in a series of

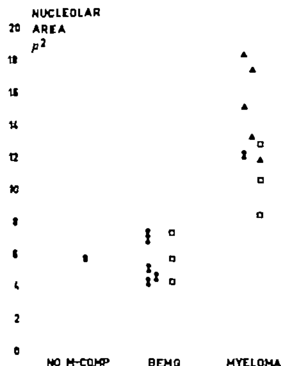


Fig 2. Mean nucleolar area in bone marrow plasma cells in myeloma, BEMO and various diseases without an M-component. ● = no cytostatic therapy ▲ = after institution of cytostatic therapy □ = BEMO developing into myeloma, no cytostatic therapy

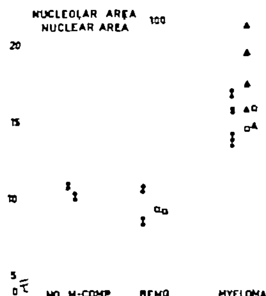


Fig 4. Nucleolar area as percentage of nuclear area in *bone marrow plasma cells in myeloma, BEMO and various* diseases without an M-component. Symbols as in Fig. 2.

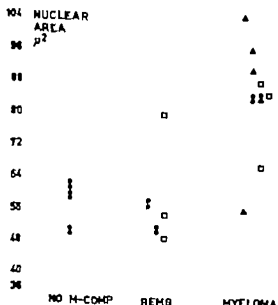


Fig 3. Mean nuclear area in myeloma, BEMO and various diseases without an M-component. Symbols as in Fig

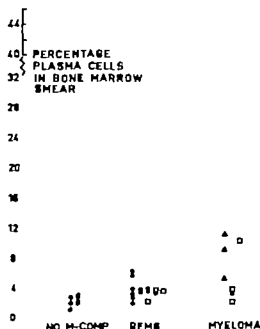


Fig 5. Plasma cell percentage in bone marrow smears in myeloma, BEMO and various diseases without an M-component. Symbols as in Fig 2.

Table IV *Nucleolar area nuclear area, relative nucleolar area and plasma cell percentage in 26 myeloma patients*

BJ = Bence Jones proteinuria, G = light chain type not determined n.d = not determined

Pat. no	M-component	Conc (g/100 ml)	BJ	Plasma cell per centage	Nucleolar area (μ^2) Mean	Nucleolar area (μ^2) S.D	Nuclear area (μ^2) Mean	Nuclear area (μ^2) S.D	Relative nucleolar area Mean	Relative nucleolar area S.D
1	GK	4.5	Trace	40	14.0	2.3	84.5	9.0	16.7	2.9
2	GK	1.6	Pos	22	12.3	3.1	79.3	14.2	15.8	3.1
3	GK	4.0	Neg.	13	14.6	3.4	84.8	12.0	17.3	3.8
4	GK	2.1	Trace	3	12.8	4.3	89.6	18.3	14.2	4.3
5	GK	0.4	Neg.	2	7.1	3.4	73.1	15.3	9.6	3.9
6	GK	1.5	n.d	10	17.8	7.1	94.3	28.9	19.0	5.8
7	GK	4.0	Pos.	27	5.9	1.4	69.9	16.9	8.5	1.8
8	GK	1.9	Trace	8	10.3	3.4	81.2	15.3	13.5	3.4
9	GK	2.3	Neg.	20	15.1	3.1	96.1	14.3	15.9	3.6
10	GK	7.0	n.d.	11	17.3	4.4	90.9	22.8	19.5	4.6
11	GK	3.2	Pos.	9	18.1	7.9	104.4	50.8	16.4	3.6
12	GL	4.8	Pos.	22	9.1	1.9	70.5	17.9	13.1	2.2
13	GL	[illegible].6	Trace	3	9.7	2.7	84.6	17.7	11.7	2.6
14	GL	6.4	Pos.	25	10.2	3.2	69.6	18.3	14.7	3.3
15	GL	6.3	Pos.	5	11.8	3.8	55.6	7.4	21.3	4.[illegible]
16	G	3.4	Pos.	8	11.2	4.5	83.5	28.5	13.6	4.3
17	G	4.7	n.d	8	8.0	1.4	66.5	13.0	12.3	2.0
18	AK	4.0	Neg.	28	14.1	3.1	80.9	15.6	17.6	2.9
19	AK	3.2	Pos.	25	12.0	4.3	75.1	12.5	15.7	1.9
20	AK	4.0	Neg.	12	11.4	4.3	85.1	25.3	13.8	1.6
21	AK	2.4	Neg.	10	14.1	4.0	96.1	19.1	14.6	3.7
22	AK	2.1	Trace	9	12.2	3.7	83.8	17.4	14.6	2.0
23	AL	1.2	Pos.	7	21.1	6.9	100.7	19.1	20.6	5.1
24	K	–	Pos	12	13.4	3.8	78.5	14.1	17.0	3.4
25	L	–	Pos.	45	12.5	3.7	60.6	19.4	21.1	3.2
26	L	–	Pos.	30	11.4	3.9	72.9	17.0	15.8	4.9
Mean		3.4		15.9	12.6		81.2		15.5	
S.D		1.7		11.5	3.5		1[illegible].0		3.2	

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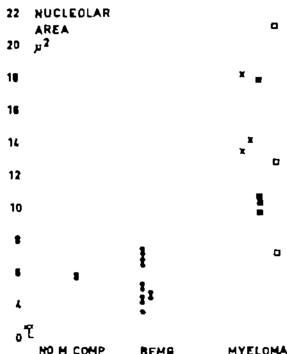


Fig 7 Mean nucleolar area in bone marrow plasma cells in various diseases without an M-component, BEMG and myeloma without osteolytic lesions at first X-ray examination. ● BEMG or no M-component, = myeloma with M-component conc. >2.0 g/100 ml *and* plasma cells in bone marrow smear >7%, ■ = myeloma with M-comp conc. ≤2.0 g/100 ml or plasma cells in bone marrow smear ≤7% □ = myeloma with M-comp conc. ≤2.0 g/100 ml *and* plasma cells in bone marrow smear ≤7%

52 consecutive cases of myeloma found no less than 17 such cases Likewise Hällén (9) found plasma cells of normal appearance in several cases of myeloma and atypical plasma cells in a few cases of BEMG Aherne (1) on the other hand who used Feulgen staining for demonstration of nucleoli found an increased nucleolar/nuclear ratio in 10 cases of myeloma as compared to 10 cases of reactive plasmacytosis

The present investigation clearly demonstrates that bone marrow plasma cells in myeloma with few exceptions differ from other plasma cells by having larger nucleoli, larger nuclei and an increased nucleolar/nuclear ratio. It can be objected that it is not possible with ordinary light microscopy to decide which plasma cells belong to the M component-producing clone and which do not. However Hijmans et al (8) have shown by immunofluorescence that in myeloma as well as in BEMG the majority of bone marrow plasma cells contain the same class of heavy and light chain as those forming the M-component The present author has been able to confirm these results and found that they are valid also in cases with a low M-component concentration and that good agreement is obtained between bone marrow aspirates from different sites in one and the same patient (unpubl shed observations).

Enlarged nucleoli and increased nucleolus/nucleus ratio are common in malignant cells. They are however not restricted to malignant cells but can be seen in growing or protein-secreting non-neoplastic cells (5 6) It has not been possible to demonstrate any consistent differences in nucleolar ultrastructure between malignant and normal cell (4) As in other blood cell series the maturation from plasma blast to plasma cell is accompanied by a gradual decrease of nucleolar size Smetana et al (14) have shown, by pecifically staining RNA containing structures that the nucleoli of normal mature plasma cells are small and have a ring-formed structure in contrast to the large and compact nucleoli of immature plasma cells Electron-microscopic studies have confirmed that ribonucleoprotein components are distributed in the periphery of such ring-formed nucleoli but uniformly in compact nucleoli. The presence of large and compact nucleoli in myeloma cells with well developed rough endoplasmic reticulum has been interpreted as a sign of maturation asynchrony of these cell components (13)

The structural observations can be viewed in conjunction with autoradiographic studies demonstrating a very low degree of RNA synthesis in normal mature plasma cells but a varying degree of persistent RNA synthesis in a high proportion of myeloma cells (11 12) Furthermore, studies of aminonucleoside inhibition of ribosomal RNA synthesis in HeLa cells have led to the conclusion that the shape and size of the nucleolus are determined by the balance between the rate of the production of the precursor of ribosomal RNA by the nucleolar regions of the chromosome and the demand for ribosomal RNA by the cytoplasm (15) Quaglino et al (11), in their autoradiographic studies of myeloma cells and normal plasma cells found no evidence of a higher degree of protei synthesis in myeloma cells In addition they observed that only a small proportion (2–10%) of the myeloma cell population did synthesize DNA

In most cases estimation of nucleolar and nuclear area does not give further information of vital importance for the diagnosis than the combination of serum and urine protein electrophoreses radiological examination of the skeleton, and examination of Pappenheim-stained bone marrow smears There are however some cases where the observation of enlarged nucleoli can make an earlier diagnosis of myeloma possible Fig. 7 shows a number of cases in which the absence of osteolytic lesions at X-ray examination, a low plasma cell percentage in bone marrow smear or a low M-component concentration initially made the diagnosis of myeloma uncertain. All but one of these cases had enlarged nucleoli when the M-component was discovered and all subsequently developed all criteria for the diagnosis of multiple myeloma.

The present material is too small to allow any definite conclusion as to whether nucleolar area has any prognostic value in myeloma. It should be pointed out, however that the 5 cases of myeloma with nucleolar areas less than 10 μ all had remarkably long survival times Two of them are still alive with observation periods of 82 and 89 months and the three who died had survival times of 71 73 and 75 months respectively The mean survival time for the whole material was 42 months

Three cases were observed in which the onset of ...loma was preceded by a long period of essen... unchanged M-component concentration dur... 5–10 years These cases should be regarded as ...ses of BEMG being transformed into myeloma. The M-component remained the same type after transformation but increased rapidly in concentration. It was not possible to sort out these cases from the other BEMG cases by measuring nucleolar area during the "benign stage When the M-component concentration increased there was a parallel increase in mean nucleolar area. This might be explained by a new clone with a higher degree of malignancy arising out of the benign clone In one case bone marrow smear was available shortly before a sudden increase of M-component concentration was observed. Measurement of a large number of cells showed an asymmetric curve with an increase in the number of cells with large nucleoli. It was however not possible to demonstrate a definitive bimodal variation.

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THE ADHESIVENESS OF HUMAN BLOOD PLATELETS AND THYROID FUNCTION

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Abstract. Hypothyroidism is associated with severe coronary atherosclerosis. In spite of this the reported incidence of angina pectoris and myocardial infarction in untreated hypothyroidism is small Since many authors consider the formation of thrombus in coronary arteries to be the final event of the process which leads to myocardial infarction, changes in the platelet function may explain the paradoxical rarity of myocardial infarction in untreated hypothyroidism To evaluate this hypothesis, platelet adhesiveness has been estimated before and after treatment in 9 hypothyroid and 16 thyrotoxic patients. In thyrotoxicosis the platelet adhesiveness was significantly increased, but decreased to normal after treatment. In hypothyroidism platelet adhesiveness was abnormally low but increased to normal value after thyroid hormone replacement. This may be an important factor in precipitating myocardial infarction in patients with hypothyroidism and coronary artery atherosclerosis.

Patients with thyrotoxicosis and hypothyroidism are in an exceptional position with regard to coronary heart disease Extensive clinical and necropsy studies have shown that severe coronary atherosclerosis occurs twice as frequently in patients with myxoedema as in controls matched for age, sex BP and associated extra thyroideal disorders (18). Despite hypercholesterolaemia and increased atherosclerosis the incidence of myocardial infarction in untreated myxoedema is not increased When thyroxin is given to such patients the incidence of angina pectoris and the risk of precipitating myocardial infarction increases (19)

In hyperthyroidism the plasma cholesterol level is low but the incidence of atherosclerosis is unknown. However an incidence of angina pectoris from 10 to 12% has been reported (3). Consequently it should be expected that a coincidental occurrence of myocardial infarction and thyrotoxicosis would not be a rarity In an unselected series of 384 patients suffering from thyrotoxicosis 7 developed myocardial infarction during an active stage of thyrotoxicosis, and in a further 8 there appeared signs of acute coronary disease suggestive of mild myocardial infarction (4). Thus the general impression of the infrequency of myocardial infarction in active thyrotoxicosis seems not to be valid

The profound influence of thyroid dysfunction on metabolic processes is well recognized but its relation to platelet adhesiveness has been sparingly investigated (16 17) The first stage in thrombus formation is the adhesion of platelets to the injured vessel wall and to each other leading to the formation of the white thrombus. As thromboembolic disorders have been reported to be associated with increased platelet adhesiveness (7 14) it seemed important to determine whether alterations in thyroid function were associated with changes in platelet adhesiveness.

PATIENTS AND METHODS

Nine hypothyroid and 16 thyrotoxic patients have been examined with regard to the adhesiveness of blood platelet by the glass bead filter method of Hellem (9) before, during and after treatment. The main principle of the method is to expose native blood to standardized glass bead column. By an electrically driven mechanical device the blood from a graduated syringe is forced through the glass bead column at constant rate. The difference of the platelet count before and after passage through the column gives the number of adhesive platelets. This is expressed as *percentage of the initial count* The normal value by this method is 75% with S.D of 13 In addition the primary bleeding time has been estimated in 6 hypothyroid patients and in all the thyrotoxic patients by modification of Ivy method (2) Normal value <12 min.

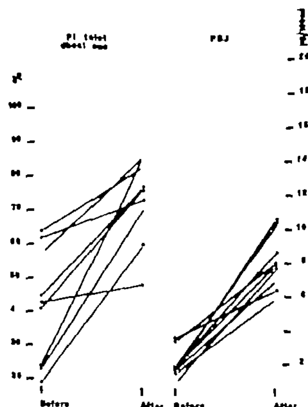


Fig 1 Percent adhesive platelets and PBJ before and after treatment in 9 hypothyroid patients.

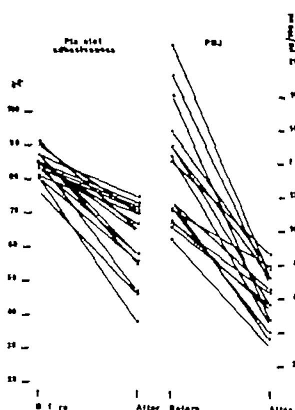


Fig 2 Percent adhesive platelets and PBJ before and after treatment in 16 thyrotoxic patients.

RESULTS

Hypothyroidism

Fig 1 shows platelet adhesiveness and PBJ before and after treatment in the 9 hypothyroid patients The mean platelet adhesiveness was 43% before and 73% after treatment This difference is highly significant ($p<0.001$) All the patients showed low initial values which increased during treatment to euthyroid state The mean platelet number was 206 000 before and 226 000 after treatment. This difference is not significant The mean bleeding time in 6 of these patients was 10 min before and 6 min after treatment ($p<0.05$)

Thyrotoxicosis

According to Fig 2 all the thyrotoxic patients showed an initial high adhesiveness which decreased to normal values during treatment to euthyroid state This is the opposite change from what was seen in the 9 hypothyroid patients The mean platelet adhesiveness was 84% before and 62% after treatment This difference is also highly significant ($p<0.001$) The mean platelet number was 231 000 before and 250 000 after treatment. This difference is not significant. The mean bleeding time remained unchanged after treatment (6½ min)

DISCUSSION

It appears that the hypothyroid patient is in some way spared from the effects of the accompanying arterial disease until thyroxin is administered The decreased platelet adhesiveness in hypothyroidism may explain the reduced incidence of myocardial infarction in spite of the extensive atherosclerotic changes in their coronary arteries However other mechanisms may also be involved in this protection. Several investigations have shown that the fibrinolytic activity of the blood is increased in hypothyroidism (1 10 11) In these reports it is suggested that the depression of the fibrinolytic activity after administration of thyroxin may facilitate the formation of an occluding thrombus in the coronary arteries In addition, decreased activity of the blood intrinsic coagulation system has been

demonstrated in the hypothyroid state (5 6). These abnormalities are reversed by administration of thyroxin. Also a slight bleeding tendency has been reported in myxoedema in spite of normal platelet count (5) indicating a qualitative platelet dysfunction.

In contrast, decreased fibrinolytic activity and increased activity of the intrinsic coagulation system *have been demonstrated in hyperthyroidism* (6 10) A slight reduction of the number of circulating platelets and a shortening of the platelet life span has also been reported indicating an increased peripheral trapping of the platelets in thyrotoxicosis (12)

From a theoretical point of view these abnormalities enchance the risk of formation of platelet thrombus. It has been claimed that the hyper thyroid patients show a low incidence of myocardial infarction (8 13) This has been attributed to the antiatherogenic effect of the thyroid hormone demonstrated in cholesterol-fed rabbits (15) Furthermore the thyroid hormones reduce the serum cholesterol level and increase the coronary blood flow which will reduce the local thrombus formation. Despite these reducing factors Burstein et al. (4) found in their series the incidence of myocardial infarction not to be less than that of a non-thyrotoxic population of analogous composition. This discrepancy may be explained by the increased platelet adhesiveness demonstrated in thyrotoxic patients.

The decreased platelet adhesiveness in the hypothyroid state may explain why these patients escape the effects of the accompanying arterial disease until thyroxin is administered. The findings reported in this paper may indicate that increased platelet adhesiveness may be a risk factor beside many others in the pathogenesis of myocardial infarction.

ACKNOWLEDGEMENT

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THALASSAEMIA MINOR

T elve P ti nts in two N rwegia Familie

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Abstract Thalassaemia minor has been diagnosed 1 members of tw Norwegian families. Haematological data from .3 family members are reported. The diagnosi based on estimation of Hb A_2 by polyacrylamide gel disc electrophoresis. All patient having high Hb A_2 levels had confirmatory evidence of thalassaemia minor (hypochromic anaemia marked aration in size and shape of the red cells, target ells, basophilic stippling, decreased osmotic fragility normal or high serum iron level). The majority of the patient had previously been treated ith iron w thout effect

The protein part of the haemoglobin molecule consists of tw separate pairs of peptide chains W th each chain is associated a haeme group The normal haemoglobins in adult life are Hb A and the minor component Hb A_2, which comprises about 2.5% of the total Hb The globin chain composition of the two haemoglobins is $\alpha_2\beta_2$ and $\alpha_2\delta_2$ respectively (7 8 20).

The thalassaemia syndromes are a group of genetic disorders in which there is a defect in the rate of synthesis of one or more of the globin chains In β-thalassaemia the most common form, the synthesis of β-chains is defecti e. The homozygous form thalassaemia major is usually a serious disease with gra e haemolytic anaemia and death in infancy or childhood In the heterozygous form thalassaemia minor or thalassaemia trait, howe er the haematological find ngs are more moderate and the clinical symptoms mild if present t all. The reduced synthesis of β-chains is associated w th an increase n the synthesis of δ-chains, consequently the most characteristic finding in thalassaemia minor is a ncreased le el of Hb A_2

The thalassaemia appear most f equently the Med terranean region and the Far East Outside these areas the disease is found in immigrant populations fr m countries whe e the disease is endemic Thalassaemia minor is however occasionally found even in aboriginal North European people (.. 4 11 13 17 18 19) In the last few years the d sease has been recognized also in Scandinavians (4 17)

The present study reveals the haematological findings in .3 members of two Norwegian families in which thalassaemia m nor was diagnosed 1 of the family members

MATERIAL

Of 25 living members of the tw families, A and B, .3 were examined haematologically The study includes 15 adult and 8 children (below 18 years) Fig 1 show the pedigrees of the tw families Both families came from the same area in the south-eastern part of Norw y near Oslo (Nedre Romerik) but no relationship between the families was know No ancestry from M diterranean, African or Asian countries was suspected.

The probands in the two families were A-III-1 and B-II 2 The former as referred because of refractory anaemia, in the latter suspicion of thalassaemia had lready been raised and he was referred for Hb electrophoresis.

Of the 12 family members who proved to be affected by thalassaemia minor (vide infra) 6 adults (A-I 1 A-III- B-I 1 B-II-2, B-II-3 B-II-4) suffered from chronic anaemia and had repeatedly been treated with iron Some also had been treated ith vitami B_{12} or liver extracts. Tw adults (A-II 3 A-II-) had been healthy without previous diagnoses of anaemia Fou children appeared to have thalassaemia Two of them (B-III-6 B-III 11) had periodically been treated with iron, one (B-III-1) had alw y looked pale whereas one (B-III-8) had gi en healthy impression None of the affected family members had received blood transfu-

Table I *Haematological findings in the family members examined (affected members given in italics)*

Pedigree no.	Sex	Age (y)	Hb (g/100 ml)	RBC × 10[6]	MCH (pg)	MCV (μm³)	MCHC (%)	Per 1 000 RBC Retic.	Target cells	Basoph. stippling
A-I-1	♀	76	11.4	4.9	23	70	32	10	26	1
A-II-2	♀	52	15.0	4.7	32	91	34	1	0	0
A-II-3	♀	51	11.9	5.1	23	73	32	11	26	1
A-III-1	♀	27	11.6	5.0	23	70	33	12	44	2
A-III-2	♀	20	12.0	5.0	24	72	33	7	16	3
B-I-1	♀	67	10.3	4.1	25	72	34	8	6	1
B-I-2	♂	67	15.4	4.6	33	100	33	10	0	0
B-II-1	♀	46	13.5	4.1	34	100	34	6	0	0
B-II-2	♂	43	11.3	6.0	19	68	28	12	12	4
B-II-3	♀	42	10.9	5.3	21	64	32	15	12	2
B-II-4	♀	40	11.8	5.7	21	65	32	10	3	2
B-III-1	♂	22	17.4	5.2	33	96	37	5	0	0
B-III-2	♀	20	13.1	4.2	31	90	35	3	0	0
B-III-3	♂	18	15.2	4.7	32	90	36	9	0	0
B-III-4	♂	16	15.8	4.6	34	98	35	1	0	0
B-III-5	♂	18	18.4	5.5	34	97	35	14	0	0
B-III-6	♀	14	11.6	5.1	23	71	32	14	3	1
B-III-7	♂	15	15.2	5.1	30	88	34	9	0	0
B-III-8	♂	11	12.0	4.7	25	78	32	13	36	2
B-III-9	♂	8	15.0	5.2	29	84	35	7	0	0
B-III-10	♂	17	14.8	4.9	30	90	34	5	0	0
B-III-11	♀	13	12.0	5.5	22	53	41	10	4	6
B-III-12	♂	4	10.9	4.9	22	70	32	10	21	9

tions, except B II-3 who had had transfusions during an operation. None gave a history of jaundice gallstones leg ulcer and none of those examined clinically had ... tory enlargement of the spleen.

METHODS

Routine haematological investigations followed standard techniques. Blood films for staining were made from blood anticoagulated with Na_2EDTA. Target cells were counted in part of the blood films where the cells were *spread in an even, single layer and in which the normal* red cells showed a clear central zone.

Osmotic fragility of the red cells was tested according to the method of Parpart et al. (16) as modified by Myhre (14).

Haemolysate for quantitation of Hb A_2 and Hb F was prepared according to Hutchinson (8) from freshly *drawn blood with heparin as anticoagulant*. 2-mercaptoethanol (final concentration 1 1000) was added to the water used for lysis of the washed packed cells. The final Hb concentration in the haemolysate was 12 13 g/100 ml.

Hb A_2 was estimated by polyacrylamide gel disc electrophoresis (Shandon apparatus) using 7% polyacrylamide gels prepared in vertical glass tubes 5×75 mm. A Tris/glycine buffer pH 8.9 (5.160 g Tris, 3.48 g glycine, 1000 ml distilled water) was used. Before application the haemolysate was diluted 1 10 in mixture of Tris/glycine buffer (1 vol.) and 25% sucrose (1 vol.) and 10 μl of this dilution (120–130 μg Hb) was applied on each gel. A current of 2 mA per tube was used until the sample had entered the gel subsequently 4 mA per tube was applied. The electrophoresis was stopped when the main Hb band had migrated 26 mm from the top of the gel. The gels were scanned unstained inside the glass tubes at 413 nm in Vitatron densitometer type TLD including an integrator.

Hb F was measured as alkali-resistant Hb by the method of Betke et al. (1) as modified by Weatherall and Clegg (20). Bone marrow studies were not included.

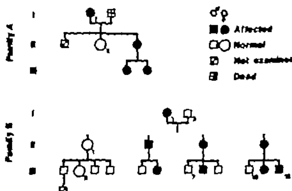


Fig 1 Pedigrees of two Norwegian families with β-thalassaemia minor

m corp. gility NaCl)	Serum iron (µg/ 100 ml)	TIBC (µg/ 100 ml)	Serum bili-rubin (mg/ 100 ml)	Hapto-globin (mg Hb/ 100 ml)	Folic acid (pg/ml) Serum	Whole blood	Serum vita-min B_{12} (pg/ml)	Hb F (% of total)	Hb A_2 (% of total)
5	100	330	0.8	110				1.5	5.2
5	105	347	0.8	86				1 9	4
6	1 1	350	0.6	64				1.8	4 6
33	155	255	0 8	20	_6	32	533	2.4	4 8
35	700	339	1 0	20	4 6	39	775	_4	7 4
38	116	285	0.4	13_		40		1_	4 8
44	112	321	0.5	100				0.5	_3
41	71	388	0.4	88				0.8	_1
36	123	300	0.5	79				2 3	6 0
38	86	408	0.6	60	4_	27	670	0 5	4 9
38	108	369	0.4	56	9	41	514	1.3	4 9
40	103	423	0.4	74				1 0	1 8
41	104	363	0.4	47				0.8	1 9
42	114	303	1.9	74				0 4	6
42	112	383	0.5	25				0.5	1.2
43	158	402	_1	100				1.3	6
38	107	427	0.8	44	6.1	61	421	1 7	6.1
43	77	420	0 7	56				1.5	1.3
34	126	339	0.6	6				0	3 3
47	88	399	0.3	6				_5	0.7
42	80	345	0.3	41				0 5	2.3
38	107	360	0 4	65	5 9	24	948	2.0	4_
33	152	363	0.6	37				1 5	6 0

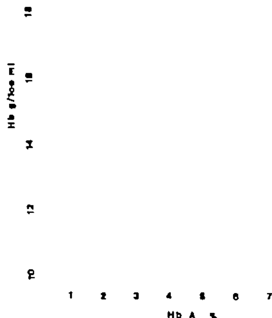


Fig. 2. Hb concentration and Hb A_2 level in the family members examined. Symbols as in Fig. 1

RESULTS

Table I summarizes the haematological data in the subjects examined. Based on the levels of Hb A_2 the family members could easily be divided into two groups (Fig. 2) and the diagnosis of thalassaemia minor could be stated by means of this estimation alone (Fig. 3). Twelve members were affected (males/females=3/9) and 11 were normal (males/females=8/3). Mean Hb A_2 value in the thalassaemia group was 5_% of total Hb (range 3 3–7 4) and in the normal group 1 9% (range 0 7–_4) (Table II). Those in the high Hb A_2 group presented outstanding morphological abnormalities of the red cells consistent with thalassaemia minor including anisocytosis poikilocytosis ovalocytosis and irregularly contracted cells while none in the low Hb A_2 group revealed such findings. Consequently there was no overlap between affected and normals concerning the Hb A_2 level.

Mean Hb F was 1.8% of total Hb in the thalassaemia and 1 1% in the normal group. There was however high degree of overlap between the two groups.

Mean Hb concentration was 11 5 g/100 ml (range 10 3–1_0) in the thalassaemia group and

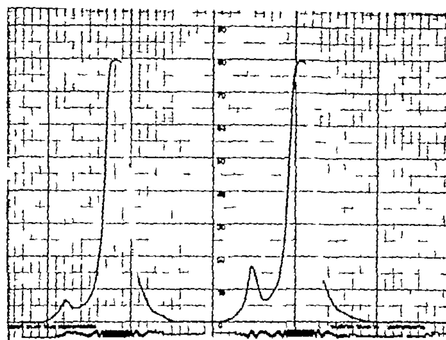


Fig. 3. Hb electrophoresis. Scanning profile of polyacrylamide gels (main peak = Hb A, minor peak = Hb A_2). *Left*: normal (B II 7), *right*: β-thalassaemia minor (A III 7).

Table II. *Summarized data from all family members (mean values, range within parentheses)*

Haematological tests	β-thalassaemia minor			Normal		
	Total	Males	Females	Total	Males	Females
Hb (g/100 ml)	11.5 (10.3–12.0)	11.4 (10.9–12.0)	11.4 (10.3–12.0)	15.3 (13.1–18.4)	15.9 (14.8–18.4)	13.8 (13.1–15.0)
RBC × 10^6/μl	5.1 (4.1–6.0)	5.2 (4.9–6.0)	5.1 (4.1–5.7)	4.8 (4.0–5.5)	5.0 (4.6–5.5)	4.3 (4.0–4.7)
MCH (pg)	23 (19–25)	22 (19–25)	23 (21–25)	32 (29–34)	32 (29–34)	32 (31–34)
MCV (μm³)	69 (53–78)	72 (68–78)	68 (53–73)	93 (84–100)	93 (84–100)	94 (90–100)
MCHC (%)	33 (28–41)	31 (28–32)	33 (3.–41)	35 (33–37)	35 (33–37)	34 (34–35)
Retic./1 000 RBC	11 (7–15)			6 (1–14)		
Se Fe (μg/100 ml)	125 (86–200)	133 (123–152)	122 (86–200)	99 (71–158)	102 (77–158)	93 (71–105)
TIBC (μg/100 ml)	345 (255–427)	334 (300–363)	347 (255–427)	381 (303–463)	375 (303–423)	397 (347–463)
Bilirubin (mg/100 ml)	0.7 (0.4–1.0)			0.8 (0.3–2.1)		
Haptoglobin (mg Hb/100 ml)	58 (6–132)			65 (6–100)		
Hb F (% of total)	1.1 (0.4–3.8)			0.6 (0.3–1.0)		
Hb A_2 (% of total)	5.– (3.3–7.4)			1.9 (0.7–2.4)		

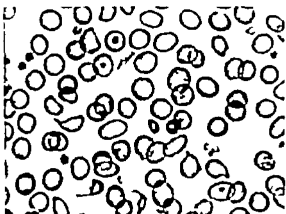


Fig. 4. Peripheral blood film of patient with β-thalassaemia minor (A-III-1) showing typical changes, including target cells.

15.3 g/100 ml in the normals. Within the thalassaemia group there was no correlation between Hb concentration and Hb A_2 level (Fig.)

Table II shows summarized additional data. RBC was moderately increased in the thalassaemia group. MCH and MCV were low in all affected members, while the overlap of MCHC values between the thalassaemic and the normal members was considerable. Reticulocyte counts were within the normal range.

Target cells were easily detected and basophilic stippling was recognized in all subjects with thalassaemia minor (Fig. 4). Osmotic fragility w s invariably decreased in this group. Fig. 5 shows a typical result of the osmotic fragility test in an affected member (B-III 1).

The serum iron level was high in 4 and w thin the normal range in the remainder of the thalassaemia group. None of the affected members had hyperbilirubinaemia, whereas 2 n the normal group, both 18-year-old males (B-III 3, B III 5), had increased serum bilirubin values without ny laboratory evidence of haemolysis or liver disease. The findings may represent some type f inherited abnormal ty of bil rubin metabolism. The 4 subjects with high serum iron level (A III 1, A III, B-III-8, B-III 1) also had low haptoglobin lues. One of them (A-III 1) ha been followed for more than one year and the haptoglobi val es ha invariably been low (10–20 mg Hb/100 ml). She also had moderately lowered folic acid values (Table I). Correction of this deficit by folic acid did not, however, affect the Hb concentration.

DISCUSSION

Opinions differ as to the severity of anaemia in the heterozygous state of β thalassaemia. According to Weatherall and Clegg (20) the majority of β thalassaemia carriers are not anaemic. Several reports, howe er, have demonstrated a significant reduction of the Hb concentration in patient heterozygous for β-thalassaemia (3, 11, 17, 18). In the present study all affected family members were anaemic.

β-thalassaemia minor usually presents as a moderate hypochromic anaemia, and the red cell morphology may be similar to that of iron deficiency. Most frequently there is a slight compensatory erythrocytosis with moderately raised RBC. These features were all present in the affected members. An outstanding feature was also the abnormalities in red cell morphology, consisting in marked variation in size and shape, and easily detectable basophilic stippling and target cells. These morphological changes were apparently not correlated to the Hb concentration or the Hb A_2 level.

A haemolytic component is commonly partially responsible for the anaemia in thalassaemia minor (8). In our study only 4 of the 12 affected family members presented evidence of increased haemolysis (low haptoglobin and high serum iron levels) but none had reticulocytosis or bilirubin concentration above normal or a history of jaundice. Characteristically, resistance to osmotic lysis was constantly increased.

In contrast to the hypochromic anaemia, the serum iron level is normal or raised in thalassaemia minor. I spite of this such patients are not infrequently treated massi ely w th iron (11, 17). Such treatment is unnecessary and potentially harmful. It should be noted, however, that these patients may de elop ron deficiency from the same causes as others (dietary deficiency, chronic intestinal bleeding, pregnancy, etc.) and they consequently need iron.

Because of compensatory erythrocytosis, patients w th thalassaemia minor may suffer from folat d ficienc particul rly in pregnancy, I which such deficiency is prone to be more marked n thal ssaemia patients than i healthy pregna

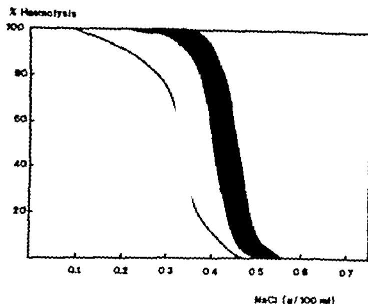


Fig 3 Osmotic fragility test from a patient with β-thalassaemia minor (B-III 17). Dark area indicates the normal range

women. These patients should therefore be carefully supervised during this period with special attention to folate deficiency and evidence of a negative iron balance (5 15). Occasionally transfusions are necessary during pregnancy

Thalassaemia minor should be suspected in patients with chronic hypochromic anaemia with normal or high serum iron level particularly if there is a family history of chronic anaemia The heridi- type of sideroblastic anaemia may be an e diagnosis However estimation of the A_2 amount by Hb electrophoresis will confirm the diagnosis of thalassaemia minor A raised Hb A_2 level can be considered virtually diagnostic of this disorder (6) If the thalassaemia is associated with iron deficiency the Hb A_2 level may be depressed, and the diagnosis consequently missed (9 10) Another estimation should therefore be made after correction of the iron deficiency

Hb electrophoresis may be performed in various media most frequently starch gel and cellulose acetate are used The polyacrylamide gel disc electrophoresis applied in this study appeared to be a suitable method The Hb A_2 values obtained were in accordance with those found in other series (6 11 12 17 18)

It may be suggested that the increasing element of immigrants and workers from the Mediterranean countries and Asia will focus the interest upon thalassaemia minor in the differential diagnosis of refractory anaemia. In North Europeans the disorder obviously is uncommon. The incidence is not known, however since no screening studies have been performed As case reports are accumulating, the recognition of the disease will probably increase

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CHANGES IN CERTAIN IRON METABOLISM VARIABLES AFTER A SINGLE BLOOD DONATION

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Abstract Signs of iron deficiency have been studied after the first blood donation in 11 healthy men. Six were given 100 mg iron daily and five received placebo tablets The total iron-binding capacity and iron absorption remained raised for more than 26 days, but had almost returned t the initial values after 70 days. A significant decrease in the stainable bone marrow iron could be shown in all subjects after 26 days, later some restitution was seen in subjects given iron supplements, but not in those given placebo As the restitution times are long, the interval after blood donation must be taken into account when judging iron metabolism variables in active donors.

Loss of blood causes changes in the haemoglobin serum iron stainable bone marrow iron, total iron-binding capacity and iron absorption from the intestine. It is known that most blood donors in Sweden show these signs of iron deficiency In order to evaluate the effect of prophylactic measures the development of signs of iron deficiency needs to be elucidated Blood donors have depleted iron stores and t is of interest to establish whether or not the iron stores are utilized in the generation of haemoglobin (10 14 20 27)

MATERIAL AND METHODS

The series comprised 12 conscripts aged 19–22 years. Six were *given 100 mg iron* as ferrous carbonate (17) daily and six received placebo tablets All were found to be fit on examination before call-up and none gave history of haemorrhage or of previous blood donation. After the start of the study one man in the placebo group refused further laboratory investigations and was excluded

Tablets were supplied for only three or four days at time. The men were asked at each visit whether they had taken all the earlier tablets, and they affirmed this Medication was stopped for fl da before each iron absorption test to wd the blocking effect of orally administered iron on the intestinal iron absorption (6, 9 11 16) Otherwise tablet ere given ntinuously throughout the stud

The *haemoglobin* (Hb) concentration was me sured as oxyhaemoglobin in Leitz n colorimeter ith filter 540 nm Triplicate samples were taken w th th su ject standing. The *serum* *total iron-binding capacity* (TIBC) and *iron absorption* ere measured described elsewhere (13 15 19 28) *Sideroblast* were counted bone marrow smears tained by the technique of Hansen and Weinfeld (17)

Stainable bone marrow ir wa semiquantitati el assessed in bone marrow squash preparations and described by Hansen and Weinfeld (1) and stained b the method of Rath and Finch ( ) T summet made estimations of 15–20 bone marrow fragment in each of two slides from each ternal puncture all on the same day The slides were marked w th code numbers, making it impossible for the examiners to iden tify them. The histochemical grades were defined as described elsewhere (8, 19 21 23).

The initial values for all ariables to be studied were *measured, all subjects gave 420 ml blood and the* iron studies were repeated during the following 14 weeks. The Hb concentration as measured daily during the first week, and subsequently twice weekly Other variables were measured on an verage 11 4 days (range 8–14) 26.3 days (range 22) and 70 3 da (range 60–82) after the blood donation The la t diagnostic iron absorption test was done only in the iron group"

RESULTS

In the *iron group* the greatest fall in Hb as noted after three days and the initial Hb concentration was regained after 30 day (Fig 1). The serum iron values showed con siderable individual variations and no conclusions can therefore be drawn.

Table I *Iron group Initial values for serum iron TIB[C] bone marrow iron and diagnostic iron absorption test and changes at 11 26 and 70 days after a first blood donation*

Tests of significance (one-sided sign test) on mean changes for the 11th and 26th day measurements, serum iron not significant, TIBC $p<0.02$, stainable bone marrow iron $p<0.02$ iron absorption $p<0.02$

	Serum iron (μg/100 ml)				TIBC (μg/100 ml)				Stainable bone marrow iron (histochemical grades)				Diagnostic iron absorption test (%)			
	Initial	Change at			Initial	Change at			Initial	Change at			Initial	Change at		
Subj. no	value	11 d	26 d	70 d	value	11 d	26 d	70 d	value	11 d	26 d	70 d	value	11 d.	26 d.	70 d
1	171	−78	−84	+ 2	378	+65	+75	+3	4	−1	−2	−0.5	48.2	− 2.4	+76	−14.7
	75	+ 9	+49	−13	403	+56	+31	+13	4	−1	−0.5	−0.5	35.1	+39.9	+33.8	− 1.8
3	87	+8	+87	+51	360	+59	+55	+ 2	3–4	0	−0.5	−0.5	10.6	+55.3	+5.0	+31.9
4	123		−35	− 1	397	–	+25	+5	4	−0.5	−2	−1	32.6	+43.0	−70.8	− 5.1
5	95	−17		− 3	369	+40		+ 3	4	0	−0.5	−1	43.7	+29.3	(−38.4)	+14.1
6	134	18	−24	+24	326	+46	−10	−16	2	−0.5	−1.5	−0.5	35.9	+21.0	+11.4	+ 4.3
Mean ±S.E.M	114±15	−15	− 1	+10	372±11	+53	+35	+ 8	3.6±0.8	−0.5	−1.2	−0.7	34.4±5.3	+31.1	+20.5	+ 4.8

Table II *Placebo group Initial values for serum iron TIBC stainable bone marrow iron and diagnostic iron absorption test and changes at 11 26 and 70 days after a first blood donation*

Test of significance (one-sided sign test) on mean changes for the 11th and 26th day measurements, serum iron not significant, TIBC p near 0.03 stainable bone marrow iron p near 0.06, iron absorption not significant

	Serum iron (μg/100 ml)			TIBC (μg/100 ml)			Stainable bone marrow iron (histochemical grades)				Diagnostic iron absorption test (%)		
	Initial	Change at		Initial	Change at		Initial	Change at			Initial	Change at	
Subj. no	value	11 d	26 d	value	11 d	26 d	value	11 d.	26 d	70 d	value	11 d	26 d
7	109	− 12	−27	372	0	+37	5	0	−2	−1.5	36.6	(−34.1)	− 6.9
8	102		+86	375		+71	3–4	–	−0.5	−1.5	35.0	–	+25.4
9	170	−108	−56	378	+13	+62	3–4	0	0	−1.5	30.7	− 8.7	+ 7.1
10	132	+ 19	− 4	391	+59	+37	3	−2	−2	−2	66.6	+ 6.8	+11.5
11	205		−55	350	–	+22	3–4	−0.5	−1.5	−2	34.8	+27.9	+12.7
Mean ±S.E.M	144±19	− 34	−11	373±7	+24	+46	3.7±0.8	−0.6	−1.2	−1.7	40.7±6.5	+ 8.6	+10.0

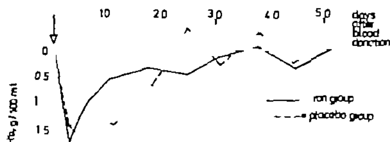


Fig 1 Mean decrease in Hb concentration after first blood donation in subjects given 100 mg iron or placebo tablets once daily Values from two samplings are combined thus, day 4+5 days 6+7 1.5 weeks, etc

The TIBC increased significantly the mean increase after 11 days being 53 μg/ 100 ml and after 26 days 35 μg/100 ml. After 70 days the values had returned to the initial levels in most subjects (Table I).

The diagnostic iron absorption test showed a statistically significant change (mean increase 31% after 11 days and 20.5% after 26 days) One subject (no. 5) was not strictly fasting when the 26th day test dose was given, and this value was therefore omitted. At the fourth measurement, 70 days after the blood donation the iron absorption had returned to approximately the initial level in most subjects (mean Increase +4.8% Table I).

The amount of stainable bone marrow iron showed a slight fall after only 11 days after 26 days the mean reduction was 1 histochemical grade which is statistically significant. A return towards initial levels then took place but no subject showed a fully restored bone marrow iron level at the time of the last examination 70 days after the blood donation.

In the *placebo group* the Hb was lowest five days after the blood donation, and had regained its initial concentration after 25 days (Fig 1). After 26 days a statistically significant TIBC increase (mean 46 μg/100 ml) was seen and all subjects but one showed increased iron absorption as compared to the initial values (Table II)

The amount of stainable bone marrow iron was reduced in all subjects but one 26 days after the blood donation. In contrast to the iron group the decrease persisted the difference being statistically significant at the last examination 70 days after the blood donation

With one exception, when poor quality smears made counting impossible sideroblasts constituted 13% or more of the erythroblasts in all bone marrow samples even in the placebo group as early as 11 days after the blood donation.

DISCUSSION

Iron stores and haemoglobin regeneration. Full restitution of the Hb concentration took 25–30 days which is in accordance with the findings of Wadsworth (25) and Alstead (1) In persons who have given blood repeatedly (4 5 7 22 24 26) and who may well have had depleted iron stores, the Hb regeneration time is longer It would therefore appear that storage iron can be used for Hb formation. This is also suggested by the reduction in the stainable bone marrow iron found in the present study after a blood loss as small as 520 ml (blood donation 420 ml plus two 50 ml samples for serum iron and TIBC estimation)

Iron for Hb regeneration after a blood loss may also be derived from absorbed iron (14) but in our iron group the Hb restitution time is no shorter than among the men receiving placebo Moreover the decrease in storage iron during the first 26 days after a blood donation, i.e the time when Hb is reforming, was similar in both groups. This again suggests that, under the conditions of the study the main source of iron for Hb regeneration was the iron stores even when supplementary iron was given by mouth.

Persistence of iron deficiency signs Increased absorption was demonstrable for more than 26 days which is a little longer than previous studies have indicated (3 27) The TIBC increase persisted for more than 26 days which is also a little longer than previously shown (18)

Signs of iron deficiency thus remain for a considerable time after single blood donation. If donors gi e blood every two months, which is not unusual in Sweden they can be expected to show signs of iron deficiency for most of that time.

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IRON SUPPLEMENT TO BLOOD DONORS

I Trials with Intermittent Iron Supply

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Abstract. A series of 58 male blood donors has been studied with regard to stainable bone marrow iron, desferrioxamine test, sideroblasts, total iron-binding capacity, serum iron and haemoglobin values. With one conventional blood donation every second month the storage iron was found to be significantly decreased after four blood donations both when placebo and oral ferrous iron in doses of up to 2000 mg were given over a period of two weeks after each blood-letting. The iron state in donors given 2000 mg was superior to that in donors given 1000 mg, and better in the latter group than when placebo was given. A moderate increase in the total iron-binding capacity could be discerned in subjects treated with placebo or only 1000 mg iron after each donation, but no changes in serum iron or haemoglobin were noted. A smaller series of 13 donors was also investigated after six donations and was found to show essentially the same pattern in the iron state variables as after four donations. When the interval between donations was four months and 2000 mg ferrous iron was given over two weeks after each donation, all variables including the amount of bone marrow iron and the desferrioxamine test remained unchanged in ten subjects after four donations. Two subjects showed a moderate decrease in the stainable bone marrow iron but it did not disappear completely.

Blood donation implies loss of iron from the donor. Although blood donors relatively seldom become patently anaemic (2, 3, 13, 14, 20), they are often found to have markedly reduced storage iron (5, 9, 19). Reduced iron stores and increased total iron-binding capacity but no other signs of iron deficiency were found in 60 regular male blood donors bled five times yearly and supplied with 600 mg ferrous fumarate after each blood donation (15). The present investigation was undertaken in order to find a model for iron supply that will compensate also for the loss of storage iron.

Two different blood donation schedules were studied: in one (study A) the donation interval was two months, in the other (study B) four months. In study A the donors received 2000 or 1000 mg iron or placebo after each donation, and a control group was included. In study B all subjects donated blood and were given 2000 mg iron after each donation.

METHODS

Haemoglobin (Hb) concentration was measured in study A as oxyhaemoglobin, in study B as cyanmethaemoglobin. In both studies a commercial Hb solution (Acuglobin, Ortho) was used for standardization. Duplicate samples were taken from venous blood drawn under light stasis after the subjects had been lying down about 10 min (initial and final examinations) or at the end of a blood donation.

Samples for *serum iron* and *total iron-binding capacity* (TIBC) were taken simultaneously with the Hb samples. The subjects were not fasting, but all sampling was done at the same time of day (1–3 p.m.). The analyses were performed in a Technicon AutoAnalyzer using the methods of Young and Hicks (21) and Henry et al (11). In study B the analyses were modified as described by Babson and Kleinman (1).

Stainable bone marrow iron was evaluated semiquantitatively in bone marrow squash preparations stained as described by Rath and Finch (17). Two examiners made separate assessments of two coded slides from each sternal puncture using a 6-grade scale 0–5 (16). In certain cases half-grades were employed, i.e. when both examiners had difficulties in placing their estimates within one of two adjacent grades or when the difference was 1 histochemical grade. The correlation between the gradings was good, in only two pairs of slides did the differences exceed 1 histochemical grade.

Sideroblasts were counted in bone marrow smears stained as described by Hansen and Weinfeld (8).

Table I *Study A. Peripheral blood variables Initial values (mean ± S D)*

Group		Hb (g/100 ml)	Serum iron (μg/100 ml)	TIBC (μg/100 ml)
Controls	13	14 4 ± 0.73	96 ± 19	–
2 000 mg	12	14.5 ± 0 42	106 ± 18	385 ± 21
1 000 mg	*12*	*14 4 ± 0 75*	*99 ± 27*	*399 ± 28*
Placebo	18	14.6 ± 0.90	103 ± 22	373 ± 32

Desferrioxamine test (DF test) was performed as described by Hallberg and Hedenberg (6) with i. injection of 10 mg desferrioxamine/kg b wt. Basal excretion was measured in sample obtained immediately before the injection, and urine was collected for 16 hours.

STUDY A

Blood Donations Every Second Month

The study embraces four groups of subjects three of which served as blood donors, each individual having been supplied with 2 000 or 1 000 mg iron or placebo after each donation, the fourth group served as controls. The study has been published earlier in Swedish (4) and a summary is given here

All subjects were young conscripts at the same regiment. All were healthy with no past history of haematological, gastrointestinal or renal disorder None had had previous haemorrhage or had served as a blood donor

Eighty volunteers were allocated at random to one of four groups. During the course of the study 22 of subjects were transferred to other regiments and did not fulfil the experiment. Three members of the 2 000 mg group returned at the end of their period of service: they were examined after six but not after four blood donations. No other subjects withdrew from the investigation The mean age of the 58 subjects completing the experiment was 20 years (range 18–23).

Design of the study The blood donors gave blood every second month. Hb serum iron and TIBC determinations were done before the first and then at the end of every donation. The blood loss on each occasion including samples was 450 mL Hb serum iron and TIBC measurements, bone marrow examination, and DF test were done in all subjects *after four donations*. Four or five members of each group were reexamined after six donations; three members of the 2000 mg group were examined only after six donations.

The interval between the fourth donation and the final investigation varied in the 000 mg group between 7 and 30 days (mean 19) in the 1000 mg group between 5 and 90 days (mean 37) and in the placebo group between 2 and 93 days (mean 42). The interval after the sixth donation, similar in all groups, was 3–18 days (mean 9.5)

After each donation two groups were given iron tablets (Ferromyn S® containing 37 mg iron as ferrous succinate and 0 11 g succinic acid). The 2 000 mg group received two tablets twice daily for two weeks and the 1000 mg group one tablet twice daily The placebo group received two placebo tablets twice daily The subjects were questioned about tablet-taking at every blood donation and at the final investigation. Some admitted not taking every tablet but not more than 10 tablets were "forgotten" by any subjects during the whole period

The control group gave no blood and no iron was administered Hb serum iron and TIBC measurements were done at times corresponding to the donor groups second donation

RESULTS

The initial values for Hb serum iron and TIBC are given in Table I Owing to a mishap TIBC was not measured in the control group

Table II *Study A. Iron state at and after the fourth blood donation Concerning Hb serum iron and TIBC differences in individual values between the fourth and first donations are given*

		Difference in mean values between the fourth and first donations			After the fourth donation		
Group		Hb (g/100 ml)	Serum iron (μg/100 ml)	TIBC (μg/100 ml)	Sideroblasts, subjects with subnormal counts[a] (% of whole group)	Stainable bone marrow iron (histochemical grades, median value)	DF test[c] (mean ± S.D) (μg/kg b.wt./16 h)
Controls	13	+0.29 NS	+28 NS	−24 NS	0	4	7.56 ± 1.55
2 000 mg	12	+0.59 NS	+29 NS	+17 NS	25	2.5	6.76 ± 1.95
1 000 mg	12	+0.50 NS	+ 9 NS	+36	42	1	5.30 ± 1.59
Placebo	18	+0.35 NS	− 8 NS	+30*	43[b]		4.82 ± 1.09

NS = insignificant difference ($p > 0.05$), * $0.01 < p < 0.05$

[a] 12 % or less. [b] One subject had given blood less than ten days before sternal puncture.
[c] Student's t-test: controls compared with 2000 mg group NS 1000 mg group $0.001 < p < 0.01$ placebo group $p < 0.001$

Table III. *Study A. Iron state at and after the sixth blood donation. Concerning Hb, serum iron and TIBC differences in individual values between the sixth and first donations are given*

Group		Difference in mean values between the sixth and first donations: Hb (g/100 ml)	Serum iron (µg/100 ml)	TIBC (µg/100 ml)	After the sixth donation: Sideroblasts, subjects with subnormal counts[a] (% of whole group)	Stainable bone marrow iron (histochemical grades, median value)	DF test[c] (mean ± S.D.) (µg/kg b.wt./16 h)
Controls	5	+0.40 NS	+47 NS	+ 1 NS	0	4	7.61 ± 1.7
000 mg	7	+0.72 NS	+17 NS	+37 NS	29	0.5	6.27 ± 1.22
1 000 mg	4	+0.90 NS	−21 NS	+42 NS	75[b]	0	4.37 ± 1.53
Placebo	5	+0.54 NS	+14 NS	+38 NS	80[b]	0	4.94 ± 1.61

NS = insignificant difference ($p > 0.05$).

[a] 12% or less. [b] Two subjects had given blood less than ten days before sternal puncture. One subject had given blood less than ten days before sternal puncture.

[c] Student's *t*-test: controls compared with 000 mg group NS, 1000 mg group NS, placebo group $0.01 < p < 0.05$.

The iron state after four and six blood donations is summarized in Tables II–IV. Both after four and six blood donations less stainable bone marrow iron was found in all donor groups than in the controls, and less in the 1 000 mg and placebo groups than in the 000 mg group (Table IV). The difference is statistically highly significant.

The DF test values were higher in the controls than in the donors, and the smaller the dose of prophylactic iron the lower the iron excretion. After six donations the mean DF value in the 1000 mg group is numerically lower than after four donations, but the difference in comparison with the controls does not reach statistical significance, probably due to the smaller number of subjects investigated after six donations.

Subnormal sideroblast counts were found in all donor groups, the 1000 mg and placebo groups showing the largest number of subnormal counts. In four subjects the subnormal count may be due to the short postdonation interval (9, 16).

Statistically significant increases in mean TIBC were found in the 1000 mg and placebo groups at the fourth donation compared to the first. The increase at the sixth donation was numerically equal and was at this point also seen in the 000 mg group. The differences at the sixth dona-

Table IV. *Study A. Classification of individuals in each group with regard to the amount of stainable bone marrow iron*

Stainable bone marrow iron (histochemical grade)	After the fourth donation: Controls (n = 13)	2 000 mg (n = 12)	1 000 mg (n = 17)	Placebo (n = 18)	After the sixth donation: Control (n = 5)	000 mg (n = 7)	1 000 mg (n = 4)	Placebo (n = 5)
4–5	1							
4	8	2	1		3			
3–4	3		1					
3	1	4	1	1				
2–3								
2		3	1	4		1		1
1–								
1		3	7	7			1	1
0–1				1			1	
0			1					3

Mann-Whitney *U*-test (18) after the fourth donation: controls compared with 000 mg group $p < 0.002$, 1 000 mg group $p < 0.001$, placebo group $p < 0.001$. After the sixth donation: control compared with 000 mg group $p < 0.001$, 1 000 mg group $p < 0.01$, placebo group $p < 0.01$.

Table V *Study B Mean Hb serum iron TIBC and DF test values before the first and 15 weeks after the fourth donation (mean±S.D)*

	Hb (g/100 ml)	Serum iron (μg/100 ml)	TIBC (μg/100 ml)	DF test (μg/kg b wt./16 h)
Initially	14 78±1 14	111±27	318±39	7.25±1 79
After 4th donation	14.88±0.92	119±35	323±40	7 09±1 72
Difference	+0 10 NS	+8 NS	+ 5 NS	−0.16 NS

Student s t-test NS=not significant.

tion do not reach statistical significance probably due to the small number of subjects.

Although initial values for DF test and stainable bone marrow iron are lacking comparison with the control group makes it seem very likely that a decrease in storage iron had already taken place after four blood donations in all donors Not even doses of 2 000 mg ferrous iron after each blood donation seem to have been enough to maintain the iron stores

STUDY B

Blood Donations Every Fourth Month

The subjects were 15 men offering blood at the Blood Centre One had had a peptic ulcer 15 years previously but no others gave any history of bleeding or had given blood All claimed to be fit and not to have ...fered from any haematological or renal disorder ... examination before registration no abnormalities ... found During the study one subject settled elsewhere and another refused further sternal punctures. Thirteen subjects aged 18–45 years (mean 37) completed the study

Design of the study Before the first blood donation Hb, serum iron and TIBC determinations, assessment of stainable bone marrow iron sideroblast counts and DF test were performed.

Four blood donations were made at mean intervals of 15 9 weeks (range 15 7–17 7). After each donation the subjects were given iron tablets (Ferromyn S® containing 37 mg iron as ferrous succinate and 0 11 g succinic acid) two tablets twice daily for 14 days i e 2 000 mg after each blood-letting. One week before each donation the iron state was checked by reexamination of all variables in part of the series (8 subjects before the second donation 7 before the third and 5 before the fourth). At 15 weeks after the fourth blood donation all subjects were reexamined All were thus investigated at least 3 times, and seven 4 times, during the study

RESULTS

The mean Hb serum iron TIBC and DF test values found initially and 15 weeks after the fourth donation are shown in Table V The differences between the initial and final values are slight and statistically insignificant.

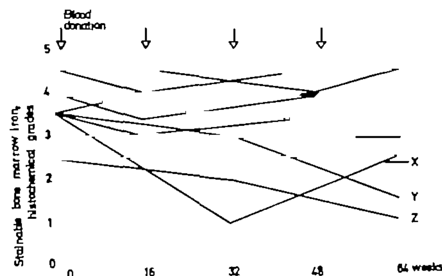


Fig 1 Study B Semiquantitative assessment of the stainable bone marrow iron in 13 subjects all of whom were examined before the first and 15 week after the fourth blood donation Subjects *X Y* and *Z* are considered separately under Results

The findings concerning stainable bone marrow iron are presented individually in Fig. 1. In 10 subjects it has remained virtually unchanged, the slight changes not exceeding 1 histochemical grade in some of the 10 subjects are probably due to errors of the method.

Subjects X, Y and Z call for comment. In X a marked decrease in bone marrow iron had taken place before the third donation. This man conceded that he had taken only a few tablets prescribed. Before the final investigation he took all the remaining tablets, the bone marrow iron increased but did not regain the initial level. In Y and Z a moderate gradual decrease is seen and the difference between the initial and final figures exceeds 1 histochemical grade. Both subjects asserted that they had in fact taken all their tablets.

Sideroblast counts were normal (≥13 %) in every bone marrow smear from all subjects.

DISCUSSION

Study A shows that the storage iron decreases rapidly when blood donors are bled every second month and when iron is not given. After four donations one third of the placebo group had no stainable bone marrow iron, all subjects but one had less bone marrow iron than the control group, the mean DF test value was significantly lower than in the controls, and 43 % had subnormal sideroblast counts.

If oral iron was given, as in the 1000 and 2000 mg groups, more storage iron remained, but the amount was significantly less than in the control group. However, the results are influenced by the interval since the latest blood donation, as a decrease in the amount of stainable bone marrow iron is demonstrable even after a single blood donation (16). Comparison with the control group ought ideally to be made using samples influenced as little as possible by the latest blood donation, e.g. samples taken immediately before or at the time of donation. The mean decrase after a single donation has been shown to be 1 histochemical grade after 26 days, and about ½ grade after 11 days. When the amount of bone marrow iron in each subject in the 2 000 mg group is corrected by +0.5 (postdonation interval 7–18 days) or by +1.0 (interval 19–30 days) the estimations represent roughly what would be expected at the preceding donation. Comparison between the bone marrow iron in the 2000 mg group (corrected as described) and the controls nevertheless discloses a statistically significant difference at the 0.05 level at the fourth, and at the 0.005 level at the sixth donation.

The DF test, which has been found to have a high correlation to storage iron as determined by chemical methods (7), parallels the results of bone marrow iron examination in this study: all donor groups excreted less iron than the control group, the difference being statistically significant for the 1 000 mg and the placebo groups.

The dissimilarity in postdonation intervals in the donor groups may bias a comparison between them somewhat, but it seems likely that in comparison to the 1000 mg and placebo groups the 2000 mg group showed a superior iron state at all events after four donations.

Essentially the same findings as in the 1000 mg and placebo groups after four donations (reduced iron stores and raised TIBC but no other signs of iron deficiency) were obtained in regular donors who had given blood 15 times and more than 50 times, all of them at a rate of five times yearly and all receiving an iron supplement of 600 mg after each donation (15). This stage of iron deficiency without anaemia thus develops rapidly in donors bled every second month, but seems not to be aggravated by further blood donation. The reason for this is probably the increased iron absorption that takes place in subjects with diminished iron stores (10, 12).

If the interval between donations is increased to four months and 2000 mg iron is given as in study B, all the variables studied including bone marrow iron and DF test remain essentially constant. This indicates that 2000 mg iron by mouth during the two weeks following each blood donation in addition to the iron in the food will be enough to compensate fully for the iron loss due to conventional blood donation in most adult men if the interval between donations is four months. The subject who did not take all his tablets demonstrates the necessity of supplementary iron in this regime. Subjects Y and Z, who showed a moderate fall ... storage iron, indicate that the chosen interval of 16 weeks is not too long as a general recommendation if the iron stores in the donors should remain unchanged.

ACKNOWLEDGEMENTS

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IRON SUPPLEMENT TO BLOOD DONORS

II. *Effect of Continuous Iron Supply*

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Abstract Seventeen conscripts gave blood every second month the amount representing an average iron loss of 3.5 mg daily Seven of them were given 20 mg and ten 100 mg iron as ferrous carbonate once daily throughout the study Before the first and after the fourth and sixth blood donations they were examined with regard to packed red cell volume, serum iron, total iron-binding capacity and stainable bone marrow iron and diagnostic iron absorption test was performed. Ten conscript receiving no iron and giving no blood but comparable to those in the test groups with regard to age exercise and diet served as controls. The stainable bone marrow iron was found to become stabilized at level with reduced but still perceptible amounts when 20 mg iron was given and at a level with somewhat greater amounts when the daily dose was 100 mg. Both levels were lower than before blood donation in most subjects, and the negative iron balance was also reflected in the diagnostic iron absorption test. When healthy person loses 3.5 mg iron daily supplementation with a 100 mg tablet per day is therefore insufficient to maintain the iron stores at their previous level. The decrease in storage iron is not progressive however When the storage iron is reduced iron absorption seems to be stimulated sufficiently to establish balance at reduced storage iron level.

Blood donors have been shown to have reduced iron stores (8 13 15 24 28 33) Many investigators therefore recommend oral iron supplementation to prevent iron deficiency in blood donors (10 17 23 26 30 3?). The efficacy of this form of prophylaxis has been questioned When as much as 2 000 mg iron was given within two weeks of each blood donation and when the interval between donations was two months the iron stores were lower in donors than in controls after only four blood donations (25). As each donation involves an iron loss of about ?00 mg less than 10% of the supplied dose seems to have been absorbed.

Since the percentage absorption of iron from the intestine decreases with increasing doses (2,5) it seemed possible that a better result might be obtained if the iron were given in smaller doses throughout the entire period between donations.

The dose was calculated as follows Six blood donations yearly (which is common in Swedish male donors) cause a total iron los of about 1?00 mg or an average loss of 3.5 mg daily To compensate for this and the physiological daily loss of 0.6 mg (9) about 4 mg/day must be absorbed by male donors Assuming the mean absorption to be 11–14% (5,27) the supplementation of the average food-iron intake of 15 mg/day (3 6 34) by ?0 mg medicinal iron per day should cover the increased loss The purpose of this study was to test the efficacy of the dose thus calculated. For comparison a large dose—100 mg daily—was also tested.

MATERIAL

Thirty conscripts aged 18–25 years, serving at the same regiment were assigned at random to two donor groups (the 20 mg and the 100 mg group) and one control group. During the study three members of the 20 mg group were posted to other regiments but none withdrew for other reasons. All had been found fit on physical examination before conscription, and none ga e history of bleeding or had previously donated blood

METHODS

The *packed cell olume* (PCV) was measured by the routine technique in duplicate samples of capillary blood spun at 18 000 g for 6 min in microcapillary centrifuge.

The *serum iron* and *total iron-binding capacity* (TIBC) were determined by routine techniques in a Technicon AutoAnalyzer using the methods of Young and Hicks (35) as modified by Babson and Kleinman (1) for serum iron, and of Henry et al. (18) for TIBC. The samples were taken in the morning, the subjects having lain down for 5–10 min and having fasted overnight.

Stainable bone marrow iron was semiquantitatively assessed in bone marrow squash preparations made as described by Hansen and Weinfeld (12) and stained by the method of Rath and Finch (31). One examiner made estimations of 15–20 bone marrow fragments on each of two slides from each sternal puncture all on the same day. *The slides were marked with code numbers, making* it impossible for the examiner to identify them. The histochemical grades were defined as follows (11 29 31): 0=no stainable iron in any of the fragments 1=small stainable granules not visible in every field (× 300), 2=small stainable granules visible in every field, 3=numerous small granules, 4=numerous large granules *5=numerous large granules with a tendency to form* aggregates 6=heavy deposits with aggregates

The *iron absorption test* was done with radioactive iron and a whole-body counter. With the subjects fasting a dose of 0.25 mg $FeSO_4$ tagged with 0.5 μC ^{59}Fe was given by mouth. The proportion of radioactivity retained in the body two weeks later is given as the result. The method, equivalent to what Heinrich (16) calls a "diagnostic" test designed to determine the degree of iron deficiency in a person, has been described in detail by Höglund (19). The test is not intended to measure the absorption from the large doses of medicinal iron given in this study

Faecal iron was tested as described by Bonnar et al. (4). ... minary tests on faeces from normal subjects taking ... carbonate pellets showed a strong positive reac... when the daily intake was 100 mg and a weak but still recognizable colour on an intake of 20 mg daily

Design of the study

The PCV serum iron, TIBC and stainable bone marrow iron were measured, and the diagnostic iron absorption test was done on all subjects at the beginning of the study. The donors then gave blood six times at mean intervals of 7.5 weeks. Reestimations of the iron variables were done after the fourth and sixth blood donations. The controls gave no blood and received no iron therapy: they were examined at the corresponding times. Radioiron for the iron absorption test was administered and samples for PCV serum iron and TIBC determinations were taken on the same day. Sternal marrow samples were obtained on other occasions. The interval between the latest blood donation and the taking of blood samples varied and will be accounted for under Results.

Iron therapy. The donor groups were given iron by mouth as ferrous carbonate stabilized with sugar in pellet form. The absorption from this particular preparation which prevents oxidation of the iron is reported to be equivalent to that from ferrous sulphate (21). The 20 mg *group received one 20 mg tablet daily* and the 100 mg group one 100 mg tablet daily throughout the study except for five days before each iron absorption test. It was strongly and repeatedly emphasized to the participants that it was very important to take the tablets. Every participant was interviewed before each sternal puncture. One member of the 100 mg group had not tolerated the tablets well and had therefore not taken all of them. One-fourth of the others admitted to having forgotten no more than 8–10 tablets during the whole period. *Faeces samples for qualitative iron analysis were brought by each subject two or three times during the study*

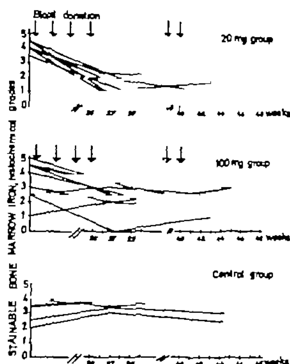


Fig. 1. Stainable bone marrow iron of different histochemical grades found initially and at various intervals after the fourth and sixth blood donations.

RESULTS

The findings initially and after four and six blood donations are shown in Fig. 1 and Table I. The intervals between the latest blood donation and blood sampling are shown in Table II.

Bone marrow examinations. The amounts of stainable bone marrow iron are presented for each subject separately in Fig. 1. Initially the mean grade was 3.4 for the controls 3.8 for the 20 mg group and 3.3 for the 100 mg group. Only 2 of 27 subjects showed a grade below 2

Table I. *PCV serum iron TIBC and iron absorption in the three groups initially and afte the fourth and sixth blood donations and differences in mean values t the penultimate nd last ex minations compared th the initial al es (d)*

	Median time since latest donation (d)	PCV (%) Mean±S D	PCV (%) d	Serum iron (μg/100 ml) Mean±S D	Serum iron (μg/100 ml) d	TIBC (μg/100 ml) Mean±S.D	TIBC (μg/100 ml) d	Diagnostic iron absorption test (%) Mean±S D	Diagnostic iron absorption test (%) d
Controls (=10)									
Initially		43.3±2.07		100±30		362±55		33.6±14 4	
After 27 weeks		42.9±1.51	−0.4 NS	101±34	+ 1 NS	359±46	− 3 NS	26.7± 9.3	− 6.9 NS
After 44 weeks		44 0±1 94	+0 7 NS	124±33	+24 NS	371±48	+ 9 NS	34.7±18.1	+ 1 1 NS
20 mg group (=7)									
Initially		43.2±2.50		98±44		362±44		24.0±13.6	
After 4 donations	43	42.3±3.34	0 9 NS	89±34	− 9 NS	377±50	+15	46.3±21.2	+22.3 NS
After 6 donations	23	41.2±1.69	−2.0 NS	75±28	−23 NS	415±58	+53	58.1±19 8	+34 1**
100 mg group (=10)									
Initially		42.1±2.04		105±33		344±46		35 9±18.7	
After 4 donations	21.5	41.3±1.86	−0 8 NS	111±48	+ 6 NS	367±33	+23 NS	52.1±22.5	+16.2 NS
After 6 donations	13.5	40.9±1 70	−1.2 NS	96±32	− 9NS	388±45	+44	56 2±15 6	+20.3

NS = not significant ($p>0.05$) $0.05<p<0.01$ ** $0.01<p<0.001$ Student *t*-test.

The control group showed essentially unchanged stainable bone marrow iron throughout the study in no subject did the change exceed 1 histochemical grade. This suggests that the Swedish army diet provides iron in adequate quantity and form

Both the 20 mg and the 100 mg groups showed a significant decrease from the initial gradings of 3.8 and 3.3 respectively to 1.6 and 2.4 respectively after the fourth blood donation. After the sixth donation the amount of stainable bone marrow iron was the same as after the fourth.

Iron absorption and variables in peripheral blood In the donor groups, but not in the controls the mean TIBC and the iron absorption as determined by the diagnostic test were found to be higher after both the fourth and sixth donations than initially The increase is statistically significant for the findings after six donations and for TIBC also after four donations in the 20 mg group. The difference was greater after six donations than after four The increases in TIBC and iron absorption were numerically greater in the 20 mg group than in the 100 mg group but the difference is statistically insignificant.

Faecal iron Samples of faeces from each donor were examined for iron on two occasions during the study together with specimens from three control subjects to serve as negative faeces controls In the 100 mg group 19 of 20 samples gave a positive reaction for iron. In the 20 mg group 9 of 14 specimens were positive The five negative samples were produced within one week of a blood donation, but subsequent samples from the same subjects showed positive reactions for iron

DISCUSSION

The high blood donation rate in this study would have practically deprived the subjects of stainable bone marrow iron after only four donations if additional iron had not been given (25) Nevertheless the mean stainable bone marrow iron decreased. Furthermore the diagnostic iron absorption test and the TIBC showed changes usually ascribed to iron deficiency Several explanations are conceivable for the latent iron deficiency that persisted despite prophylactic iron therapy

1 The iron deficiency may be only apparent, and the signs of iron deficiency may be a result of too short intervals between donations and laboratory tests The greater increase in TIBC and

Table II *Interval between latest blood donation and laboratory tests*

	After four donations (d.)		After six donations (d.)	
	20 mg group	100 mg group	20 mg group	100 mg group
Sternal puncture				
Range	8–22	8–28	19–48	16–48
Mean	12.3	15.5	32 9	27.2
Median	*9*	*16*	39	20
Absorption test and peripheral blood values				
Range	11–44	7–43	7 34	4–39
Mean	36 4	24.5	20 4	18.6
Median	43	22	24	13

iron absorption in both donor groups after six donations than after four can for example be ex plained by the shorter interval after the sixth dona tion However the very fact that the signs of iron deficiency persist for nearly one year when the mean interval between donation and test was as long as 36 4 days (Table II) suggests that latent iron deficiency is actually present most of the time.

2. Donors may have neglected to take their iron The interviews with the donors and the faecal suggest a certain neglect. Even if it is 'sumed that 15% of the tablets had in fact not been taken the 20 mg group was nevertheless receiving an average of 17 mg iron per day and the 100 mg group an average of 85 mg per day

3 There may be unsatisfactory absorption of the iron from the tablets taken. Even with the *above reservations in mind it seems difficult to* reach any other explanation. Probably less than 20% and 4% of the 20 mg and 100 mg tablets respectively was absorbed The proportion may appear low but tallies with data given by Heinrich (16) from which it can be inferred that only 4–5 mg can be expected to be absorbed from a single 100 mg dose in men with normal" iron stores In our investigation repeated doses were given and still less of the iron could therefore be ex *pected to be absorbed owing* to the mucosal block (7 20).

The latent iron deficiency found does not appear to be of a progressive nature but a balance seems to be established The fact that the iron state after six blood donations was the same as after four suggests that the iron lost at the fourth and fifth donations was regained before the next one in both groups The total quantity of iron absorbed from food and tablets must therefore have been about 4 mg/day both in the subjects taking 20 mg and *in those taking 100 mg iron daily*

The 20 mg group thus balances at a level char acterized by low storage iron and a pathological diagnostic iron absorption test the 100 mg group balances at a higher level with less abnormal values This can be explained by the inverse rela tionship between iron absorption and the size of the iron stores shown by Hausmann et al (14) and Höglund et al (22) When the stores are low a greater proportion of the iron dose is absorbed, and at a certain point balance is achieved

In conclusion 20 mg ferrous iron daily to blood donors is enough to compensate for the iron lost by blood donation only if reduced bone marrow iron stores are accepted. If larger stores are aimed at, much greater doses must be given even a dose of 100 mg dail (36000 mg yearly) seems to be too little to maintain iron stores at their predonation levels It is doubtful whether such huge doses would be realistic in blood bank practice.

ACKNOWLEDGEMENT

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DEATHS FROM ISCHAEMIC HEART DISEASE IN HELSINKI IN THE YEARS 1959–1968

Vital Statistics and Medico-legally Autopsied Sudden Deaths

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Abstract According to the official vital statistics, altogether 10910 deaths from ischaemic heart disease (IHD) occurred in persons resident in Helsinki during the 10-year period 1959–68. A significant increase was found in the incidence of IHD deaths in both sexes even though changes in the structure of the population were taken into account. The increase in the age-dependent incidence of IHD deaths was most conspicuous at middle age in both sexes and in young males. Altogether 3044 IHD deaths occurring unwitnessed or within 24 hours of the onset of the fatal attack were autopsied medico-legally during the 10-year period of the study The medico-legally autopsied cases obviously represented high proportion of sudden IHD deaths occurring outside hospitals in Helsinki. A clear male preponderance was found in the autopsy material as compared with all IHD deaths. The prevalence of cases of acute myocardial infarction varied in different years from 27 to 49% at medico-legal autopsies. No significant change occurred during the 10-year period in the distribution of the medico-legally autopsied IHD deaths into social groups, in the suddenness or place of death. The home was the most common place of death From 1963 onwards the patients dying from an ischaemic heart attack during transportation to hospital or in an outpatient department constituted 6–7% of all annual IHD deaths.

According to the clinical epidemiological prospective studies (10 12) and vital statistics (35) Finland is one of the leading countries in both morbidity and mortality from ischaemic heart disease (IHD) Studies from previous decades suggest that IHD mortality has increased continuously in Finland (6 7 30 32) Nowadays the population of Helsinki the capital of the country certainly represents a mixed Finnish population owing to brisk migration. Thus the disease situation in Helsinki may present a good cross-section of the situation in the country

Suddenness of death is without doubt, one of the most difficult problems associated with IHD The essential role played by IHD as a cause of sudden death is evident in many postmortem series varying from 50 to 91% of sudden natural deaths (14 15 28 31) A great majority of sudden IHD deaths occur outside hospitals and before any medical care is available. This was also shown in an extensive epidemiological cross-sectional and retrospective study performed in Helsinki in 1970 by the Ischaemic Heart Disease Register (25 26) The study snowed that about 80% of IHD deaths in Helsinki are confirmed at autopsy The most problematic sudden IHD deaths in Finland those occurring outside hospitals are autopsied medico-legally In Helsinki all medico-legal autopsies are performed in the Department of Forensic Medicine University of Helsinki. Since there has been no organized register system in Helsinki for the collection of the data related to IHD deaths before the Ischaemic Heart Disease Register was established and started in 1969 It is obvious that the most suitable way to obtain information on the sudden IHD deaths which have occurred during previous years is to analyse retrospectively the medico-legally autopsied cases. Since the autopsy rate is so high one can expect that these patients form a rather representative sample of sudden IHD deaths occurring outside hospitals in Helsinki.

The present study was performed in order to

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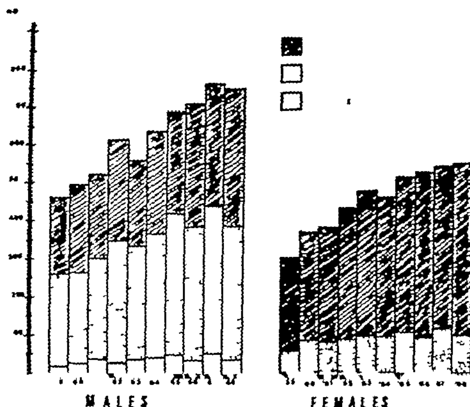


Fig 1 Deaths from IHD by age and sex in Helsinki during 1959–68

timate the history of IHD mortality in Helsinki during the 10-year period 1959–68 In addition a more detailed analysis of the medico-legally autopsied sudden IHD deaths from the same period is presented The present paper deals with the official vital statistics and certain factors related to the history of the fatal attack and social background of the medico-legally autopsied sudden IHD deaths

MATERIAL AND METHODS

Vital statistic

The results concerning IHD mortality in Helsinki are based on the official vital statistics obtained from the Central Statistical Office of Finland The principal source of this information is the death certificates gathered at the Statistical Office annually from the whole country During the 10-year period of the study the nomenclature of the diseases was based in Finland on the International Classification of Diseases from 1952. The cases in which the underlying cause of death was coded 420, IHD were taken as the object of the study

Medico-legal material

The medico-legal material consist of subjects autopsied in the Department of Forensic Medicine University of Helsinki, during 1959–68 for whom the cause of death was IHD and the death had not been witnessed or the fatal attack had terminated within 24 hours of the onset of symptoms. Only those who resided in Helsinki at the time of death were included

According to WHO rules the diagnosis of IHD death is definite at autopsy if a recent myocardial infarct and/or a fresh coronary thrombosis can be demonstrated (34). The prevalence of medico-legally autopsied cases fulfilling these criteria of acute myocardial infarction (AMI) was analysed annually on the basis of the autopsy records The diagnosis of a recent infarct was based mainly on macroscopic findings, but also in certain cases on conventional histology or on macroscopic histochemical staining of myocardial specimens (19). The histochemical method was applied to routine autopsy work in the Department during 1966–68

The division of the medico-legally autopsied subjects into three social groups was based on the subject occupation. The criteria presented by Rauhala (23) were followed the principles being in common use in Finland

The history of the fatal attack was gained from the police report in all cases and from the hospital records if the patient had been transported to a hospital or to an outpatient department.

The autopsied subject were divided into three groups according to the suddenness of the death 1) deaths occurring within 1 hour of the onset of symptoms) deaths occurring within 1 4 hours of the onset of

Table I. *Total mortality rate in relation to deaths from IHD per 1000 inhabitants per year during 1959–68 in Helsinki*

	Males			Females		
	Total	IHD	IHD/total (%)	Total	IHD	IHD/total (%)
1959	9.95	2.39	24.0	8.58	1.24	14.5
1960	10.39	2.52	24.3	8.96	1.50	16.7
1961	10.38	2.61	25.[illegible]	9.17	1.53	16.7
1962	10.42	2.98	28.7	9.78	1.70	17.4
1963	10.13	2.64	26.0	9.49	1.80	19.8
1964	10.23	2.95	28.9	9.24	1.73	18.7
1965	10.78	3.11	28.9	9.39	1.90	20.2
1966	10.32	3.12	30.3	9.30	1.89	20.3
1967	10.79	3.31	30.8	9.04	1.92	21.2
1968	10.52	3.21	30.5	8.98	1.93	21.5

symptoms, 3) unwitnessed deaths in which the duration of the terminal attack was not exactly known. Probably most deaths in the third group were also sudden, occurring at least within 24 hours of the onset of symptoms.

The place of death was grouped as: 1) home, 2) transit or an outpatient department, 3) other places (at work, in the street, etc.) including hospital departments (86 persons, 2.8% of the entire material).

RESULTS

Vital statistics

During the 10-year period 1959–68 altogether 10910 fatal attacks due to IHD were recorded among residents of Helsinki. The absolute number of IHD deaths increased during this period in all age groups (Fig. 1). The average population of Helsinki increased considerably and equally in both sexes during the same period from 442800 in 1959 to 529200 in 1968. The total mortality and the mortality from IHD per 1000 inhabitants per year are given in Table I. Only a minimal increase if any was observed during the 10-year period in the incidence of all deaths. On the other hand the incidence of IHD deaths increased 34.3% in males and 55.7% in females. The proportion of IHD deaths in relation to all deaths increased from 24.0% in 1959 to 30.5% in 1968 in males and from 14.5% in 1959 to 21.5% in 1968 in females. The age-dependent incidences of IHD deaths from 1961 and 1967 are given in Table II. The increase in the age-dependent IHD incidence was most conspicuous in middle-aged males and females and in young males being about similar in females aged 45–64 and in males aged 25–44 and 45–64 years.

Medico-legal material

During the 10-year period 1959–68 altogether 3044 fatalities among residents of Helsinki, occurring unwitnessed or within 24 hours of the onset of the fatal attack, i.e. 28% of all IHD deaths were diagnosed as IHD deaths at autopsies performed in the Department of Forensic Medicine University of Helsinki. The medico-legally autopsied cases fulfilling the criteria of the study formed an increasing proportion of all IHD deaths 19.6% in 1959 and 33.6% in 1968 (Table III). The number of medico-legally autopsied males during the 10-year period was 2009 and that of

Table II. *Age-dependent incidence of IHD deaths per 1000 inhabitants per year in Helsinki in 1961 and 1967*

	Males			Females			Total		
Age (y)	1961	1967	Increase (%)	1961	1967	Increase (%)	1961	1967	Increase (%)
25–44	0.46	0.67	45.7	0.10	0.09		0.26	0.36	38.5
45–64	5.81	8.23	41.6	1.12	1.64	45.3	2.83	4.28	51.3
>65	21.9	24.8	13.2	11.2	13.0	16.1	14.0	16.4	17.1

Table III. *Medico-legally autopsied sudden IHD deaths during 1959–68 in Helsinki the proportion of these cases in relation to all IHD deaths the prevalence of AMI cases the distribution of cases into social groups suddenness and place of death and the deaths in transit or in outpatient departments as a percentage of all IHD deahts (2/T)*

	No of cases	Proportion of all IHD deaths	Prevalence of AMI cases	Social class			Suddenness of death (group)			Place of death (group)			
				I	II	III	1	2	3	1	2	3	2/T
1959	152	19.6	77.0	13	36	51	64	22	14	61	20	19	4.0
1960	198	22.6	36.4	18	30	52	59	29	12	57	22	21	4.9
1961	700	21.6	42.0	13	35	52	54	23	23	64	16	20	3 4
196_	276	25.9	45.3	12	33	55	74	13	13	69	14	17	3.6
1963	264	25.0	32.6	14	31	55	62	15	23	53	27	70	6.6
1964	321	28.8	35.5	14	33	53	58	19	_3	60	23	17	6.5
1965	365	29.8	35 4	15	34	51	55	18	27	62	23	15	6.8
1966	384	30.6	49.0	13	32	55	58	16	26	57	23	20	6.9
1967	438	33.1	46.0	12	34	54	63	10	77	62	20	18	6.7
1968	445	33.6	48.8	16	37	52	52	34	14	64	23	13	7 7
Total	3 044	27 9	41.3	14	33	53	60	19	21	61	1	19	5.9

Division into groups, see Material and methods.

females 1035 The male female ratio was thus 1.9 1 (Table IV). According to official vital statistics the corresponding sex ratio for all IHD deaths in the 10-year period was 1.3 1 Males were overrepresented in the medico-legal material in all age groups (Table IV) The absolute number of IHD deaths was greater in males than females below the age of 65 years while a sex ratio was found at older ages both for IHD deaths and for medico-legally autopsied cases (Table IV). The age-dependent incidence of all IHD deaths per 1000 inhabitants of the same age in the Helsinki population was however clearly higher in males than in females in all age groups (Table II) About 53% of all IHD deaths among subjects below 45 years had been autopsied medico-legally while the corresponding autopsy rate was 36% in the age group 45–64 and 21% in the age group 65 and above

Table IV *Sex ratio for all IHD deaths and for the medico-legal autopsy material by age the years 1959–68 combined*

Age (y)	Total (♂/♀)	Medico-legal autopsy material (♂/♀)
≤44	6.1/1	6.7/1
45–64	3.3/1	3.8/1
≥65	0.7/1	0.9/1
Total	1.3/1	1.9/1

The prevalence of AMI cases according to WHO criteria varied in different years from 27 to 49% at medico-legal autopsies (Table III). During the last three years when the macroscopic histochemical staining of the myocardium had become a routine autopsy method in this Department the prevalence of detected recent infarcts was highest.

No significant change had occurred during the period in the distribution of medico-legally autopsied IHD deaths into the three social groups (Table III) The distribution of males into the social groups was clearly different from that of females (Table V). The estimation of the social group was probably erroneous in many females due to the high prevalence of unspecific occupations such as housewife etc No consistent difference was found in males in the distribution into social groups in different age groups The small number of females in the younger age groups makes the interpretation of the significance of the age group differences in them difficult.

The distribution of the medico-legally autopsied cases into the analysed groups of suddenness of death did not change during the period (Table III) In about 80% of these IHD deaths the fatal attack had been unwitnessed or had terminated within 1 hour of the onset of symptoms (Table VI) In both males and females unwitnessed deaths were more common after the age of 65 than at younger ages The death had been unwitnessed more often in females than in males.

Table V *Distribution of medico-legally autopsied sudden IHD deaths into three social groups (I–III) by age and sex the years 1959–68 combined (data for social group available in 2970 cases)*

	Males							Females						
		I		II		III			I		II		III	
Age (y.)	Total	*N*	%	*N*	%	*N*	%	Total	*N*	%	*N*	%	*N*	%
≤44	188	40	21	73	39	75	40	28	1	3	8	29	19	68
45–54	448	78	17	188	42	182	41	82	3	4	23	28	56	68
55–64	697	110	16	273	39	314	45	226	11	5	50	22	165	73
≥65	629	126	20	247	39	256	41	672	41	6	125	19	506	75
Total	1962	354	18	781	40	827	42	1008	56	6	206	20	746	74

The witnessed attacks terminating within 1 hour were slightly more common in males About 20% of both sexes had survived more than 1 hour after the onset of symptoms

No significant tendency to a change in the distribution of medico-legally autopsied cases into the groups of place of death was found during the 10-year period (Table III) It is noteworthy that the proportion of patients dying during transportation to a hospital or in outpatient departments was rather constant in relation to the number of all IHD deaths From 1963 these cases constituted 6.5–7 7% of all annual IHD deaths In absolute figures this signified an increasing number of patients per year from 70 in 1963 to 102 in 1968 The frequency of deaths occurring in transit or in outpatient departments was not dependent on age but was higher in males than in females (Table VII) The home was the most usual place of death more often in females than in males and more often in elderly than in younger males

DISCUSSION

The incidence of deaths from IHD and the proportion of these deaths in relation to all causes of death have increased considerably among the population of Helsinki during the 10-year period of the study The increase is valid even though the changes in the number and composition of the population are taken into account. It is noteworthy that the increase in age-dependent IHD mortality has been highest among persons of middle age. The increasing tendency to IHD mortality has also been found in several other countries (2 3 11 18) On the other hand some authors have criticized the results based on vital statistics and have suggested that there is no evidence of an increasing incidence of IHD e.g. in England and the USA and that the changes in the age structure of a population and in diagnostic habits may largely explain the increasing prevalences of the disease (24). From previous studies it is well known that mortality from IHD is highest in the eastern parts of Finland (10, 22). It was

Table VI *Prevalence (%) of subjects dying unwitnessed within 1 hour and 1–24 hours of the onset of the fatal attack in the medico-legal autopsy material during 1959–68 by age and sex*

	Males (group)							Females (group)*						
		1		2		3			1		2		3	
Age (y.)	Total	*N*	%	*N*	%	*N*	%	Total	*N*	%	*N*	%	*N*	%
≤44	188	126	67	40	21	22	12	28	21	75	4	14	3	11
45–54	450	297	66	85	19	68	15	82	44	54	21	25	17	21
55–64	717	473	66	136	19	108	15	228	128	56	48	21	52	23
≥65	654	406	62	118	18	130	20	697	327	47	140	20	230	33
Total	2009	1302	65	379	19	328	16	1035	520	50	213	21	302	29

Division into groups, see Material and methods.

Table I *Age and sex distribution of persons autopsied in pathologic or forensic departments and of non-autopsied deaths*

Age (y)	Recent changes at autopsy[a]								Clinical data			
	Pathologic departments				Forensic departments				Non-autopsied deaths			
	Total	Definite	Probable	None	Total	Definite	Probable	None	Total	Definite	Possible	Insuff data
Males												
–44	11	7	2	2	29	7	10	12	1	–	1	–
45–54	28	20	3	5	54	19	15	20	10		3	5
55–65	92	58	18	16	121	29	45	47	62	11	12	39
Total	131	85	23	23	205	56	70	79	73	13	16	44
Females												
–44	1	1	–	–	3	–	2	1	–	–	–	–
45–54	4	4	–	–	12	2	5	5	1	1	–	–
55–65	35	23	7	5	41	11	15	15	20	9	4	7
Total	40	28	7	5	56	13	22	21	21	10	4	7
Both sexes	171	113	30	28	261	69	92	100	94	23	20	51

An acute infarction or a fresh coronary thrombus.

deaths autopsied in the pathologic departments of hospitals or in the Department of Pathology University of Helsinki, were dealt with separately from those autopsied medico-legally About half of all fatal cases had been autopsied medico-legally and one third in the pathologic departments. The remaining 18 % of IHD deaths were recognized without autopsy

The reliability of the diagnosis of IHD death (Table I) determined by the instructions laid down by WHO). The categories were based in autopsied cases on postmortem evidence of an acute ischemic attack. A "definite" death from IHD was a case in which either a macroscopic necrotic infarction was found at autopsy or a fresh coronary artery thrombosis was demonstrated. The "probable category included cases in which a non-necrotic macroscopic appearance of a recent infarction was observed with the naked eye and/or infarct changes were demonstrated by histology The "none category in autopsied cases comprised those in which no evidence of an acute infarction or thrombosis could be obtained at autopsy In non-autopsied cases the diagnostic categories were based on clinical data (22). In "definite" case changes in ECG or serum enzymes were reported, the diagnosis possibly being supported by a history of typical chest pain. The "possible" infarctions were those with a typical history of chest pain in association with the attack, while ECG or serum enzyme evidence was lacking "Insufficient data" were those in which none of the above mentioned clinical evidence was obtained, but no diagnosis other than that of IHD was made Definite or probable patho-anatomic evidence of an acute heart attack was obtained in 84 % of persons autopsied in the pathologic departments and in 62 % of those autopsied medico-legally A history of typical chest pain in association with the fatal attack or of a previous IHD was known in 25 of 28 persons in the "none" category in the pathologic department series and in 70 of 100 persons in the "none" category in the medico-legal series. Definite and possible clinical categories were obtained in 46 % of non-autopsied cases. A history of a previous IHD was known in 42 of 51 persons in the "insufficient data" category Hence, the diagnosis of IHD death was either based on the positive patho-anatomic or clinical evidence of an acute heart attack or else supported by a history of clinical IHD in no less than 92 % of all registered fatalities.

The majority of deaths autopsied in the pathologic departments were delayed while most persons autopsied medico-legally had died suddenly or unwitnessed (Table II). Of 171 persons autopsied in the pathologic departments 163 (95 %) and of 94 non-autopsied persons 34 (36 %) had died in a hospital. Of 261 medico-legally autopsied persons 242 (93 %) had died outside hospitals.

Social groups

The division into four social groups was based on the person s occupation (22). The same division has been used in the census in Finland e g. by the Statistical Office of the City of Helsinki (23).

Disease history

Information on previous diseases was obtained by the interviewers from the patients or their relatives and from hospital records. The history of a previous myocardial infarction is given in this paper according to two categories, confirmed" and "non-confirmed" suggested by the WHO The history of a previous angina pectoris was based on the definition of the WHO (31) The persons were in this respect divided into those who had suffered from angina for more than 28 days before death and those whose first anginal symptoms appeared within 28 days preceding death. The level of medical care was evaluated by recording the visits to a doctor within 28 days preceding death.

Table II *Suddenness of autopsied and non-autopsied deaths*

	Total no. of pats.	Less than 1 h		1–24 h		24 h – 28 d.		Unwitnessed		Insuff data
		No	%	No.	%	No.	%	No.	%	
Pathologic departments										
Males	131	27	21	26	20	75	57	0	0	3
Females	40	3	7.5	13	32.5	22	55	1	2.5	1
Forensic departments										
Males	205	130	63	17	8	26	13	32	16	0
Females	56	30	54	9	16	7	13	10	18	0
All autopsied cases	432	190	44	65	15	130	30	43	10	4
Non-autopsied deaths										
Males	73	41	56	10	14	17	23	5	7	0
Females	21	6	29	3	14	10	48	2	10	0
Total	94	47	50	13	14	27	29	7	7	0

Visits due to premonitory symptoms were analysed separately. An attempt was made to clarify the previous medication of patients, particular attention being focused on the use of digitalis.

Autopsy data

All departments of pathology and forensic medicine in Helsinki were informed of the study. Detailed instructions were given by the Register team to the pathologists to complete a special record form whenever a fresh myocardial infarction was found or in cases when ischemic heart disease was considered to be an essential cause of death. Routine autopsy technique was employed in the Departments of Pathology Aurora, Kivelä and Maria Hospitals and in the Department of Pathology University of Helsinki. Patients dying in Meilahti Hospital, the biggest hospital in the city are autopsied in the Pathologic Department of the University. The results of histologic examinations at 29 autopsies in the pathologic departments were reported.

A number of 248 medico-legally autopsied persons had been examined in the Department of Forensic Medicine, University of Helsinki. A member of the study team carried out the examination of the hearts in 194 registered IHD deaths. A special methodology suggested by the WHO scientific group (32) was applied to the investigation of these cases, which constituted 74 % of the medico-legal series. A double contrast modification of postmortem coronary angiography was developed and used for the demonstration of changes in the coronary arteries (20). In addition, the longitudinally opened coronary arteries were carefully examined. The heart was sectioned into slices from the apex to the base. The slices were stained by a macroscopic histochemical method (19) for demonstration of infarcted areas. Myocardial blocks were taken for histology from macroscopically suspected areas or if the macroscopic examination was negative from the anterior and posterior wall of the left ventricle and from the interventricular septum. The principles adopted in the WHO Cooperative Study (33) were followed when making the histologic diagnosis of recent infarct lesion. In 67 medico-legal autopsies the patho-anatomic findings of the heart were based on routine autopsies, the record forms being completed by a member of the team.

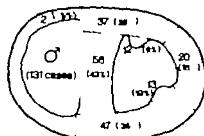
A recent myocardial infarction was regarded as definite when macroscopic necrosis was observed with the naked eye in the myocardium (34). The ruptured infarctions were recorded separately. The infarction was "probable" when discoloration, pallor or softening but no necrosis was observed in demarcated area of the myocardium and/or histologic changes of an acute infarction were demonstrated by microscopic examination. The location of an infarction was indicated by drawings (Fig. 1). A scar 0.5 cm or more in diameter was regarded as an old healed infarction (8). The location of scars was also shown by drawings (Fig. 2). A slight fibrosis less than 0.5 cm in diameter was recorded separately. The heart had been weighed at autopsy in 403 cases. The presence of fresh coronary thrombus was estimated visually. The degree of coronary stenosis was also reported in record forms. Since the estimations made by several pathologists are not truly comparable, only the reported frequencies of coronary occlusions are given.

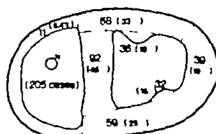
The statistical computations of this study were performed with Burroughs 6700 computer in the Computing Centre of the University of Helsinki. The statistical methods and tests used in this study are standard and commonly known.

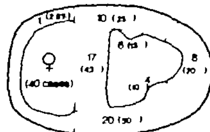
RESULTS

Social groups

All registered cases of acute ischemic heart disease (AIHD) and all autopsied cases showed a division into four socio-economic groups very similar to that of the population of Helsinki according to the census made in 1965 (Table III). Men belonging to the highest social group (I) were under-represented and those of the lowest social group (IV) over represented among medico-legally autopsied cases







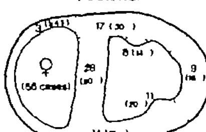
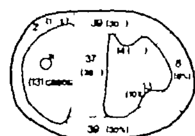
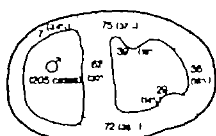
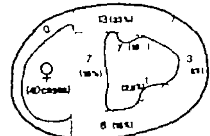


Fig 1 Prevalence of recent infarct changes in various segments of the wall of cardiac chambers in males and females autopsied in the pathologic and forensic departments.







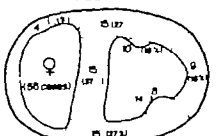


Fig 2 Prevalence of old infarct scar in various segments of the wall of cardiac chambers in males and females autopsied in the pathologic and forensic departments.

Table III *Distribution of autopsied and non-autopsied deaths and all registered cases of AIHD into four socio-economic groups and the corresponding distribution of males and females aged 15 years and above in the population of Helsinki*

	Total no. of pats.	Group I No.	Group I %	Group II No.	Group II %	Group III No.	Group III %	Group IV No.	Group IV %	Unknown No.	Unknown %
Pathologic departments											
Males	131	27	21	24	18	58	44	22	17	0	
Females	40	1	2.5	10	25	19	47.5	10	25	0	
Forensic departments											
Males	205	26	13	47	23	87	43	41	20	4	2
Females	56	5	9	9	16	24	45	15	28	3	5
All autopsied deaths	432	59	14	90	21	188	44	88	1	7	2
Non-autopsied deaths											
Males	73	19	26	21	29	24	33	6	8	3	4
Females	21	3	14	2	10	10	48	6	29	0	
Total	94	22	23	23	25	34	36	12	13	3	3
Registered cases of AIHD											
Males	953	187	20	197	21	41	44	149	16	9	1
Females	314	25	8	62	20	143	46	78	25	6	2
Population of Helsinki											
Males			19		21		43		16		0.3
Females			7		19		49		25		0.3

while among non-autopsied persons the situation was the opposite. A relatively greater proportion of acute attacks of IHD in persons belonging to social group IV had terminated fatally (44%) than in persons of social group I (38%). The difference was not, however, significant. In the social groups II and III the corresponding frequencies were 44% in the former and 40% in the latter.

Disease history

A previous infarction was known more often in men autopsied in the pathologic departments than in men

Table IV *Prevalence of the history of a previous myocardial infarction, angina pectoris more than 28 days before death and first anginal symptoms within 28 days preceding death in autopsied and non-autopsied deaths and all registered cases of AIHD*

	Total no. of pats.	Previous infarction: Data available	Previous infarction: No. of cases	Previous infarction: %	Angina>28 d.: Data available	Angina>28 d.: No. of cases	Angina>28 d.: %	Angina<28 d: Data available	Angina<28 d: No. of cases	Angina<28 d: %
Pathologic departments										
Males	131	129	76	59	127	94	74	127	7	6
Females	40	40	15	38	39	30	77	36	1	3
Forensic departments										
Males	205	162	50	31	151	94	62	149	4	3
Females	56	43	16	37	40	27	67	36	1	3
All autopsied deaths	432	374	157	42	357	245	69	348	13	4
Non-autopsied deaths										
Males	73	6[illegible]	29	47	65	54	83	58	2	3
Females	21	18	3	17	17	9	53	14	1	7
Total	94	80	32	40	82	63	77	7[illegible]	3	4
All registered cases of AIHD	1 267	1 177	425	40	1 162	802	69	1 145	[illegible]	11

Table V *Visits to a doctor and visits due to premonitory symptoms within 28 days preceding death and the use of digitalis in autopsied and non-autopsied deaths and all registered cases of AIHD*

	Visits to a doctor					Use of digitalis		
	Data available	All visits		Premonit. sympt.		Data available		
		No of cases	%	No. of cases	%		No. of cases	%
Pathologic departments								
Males	125	81	65	34	27	125	75	60
Females	37	29	78	12	32	40	26	65
Forensic departments								
Males	143	65	45	23	16	157	40	25
Females	40	19	48	7	17	43	20	47
All autopsied deaths	345	194	56	76	22	365	161	44
Non-autopsied deaths								
Males	62	39	63	15	24	63	28	44
Females	17	6	35	1	6	16	11	69
Total	79	45	57	16	20	79	39	49
All registered cases of AIHD	1 136	481	42	204	18	1 127	469	42

autopsied medico-legally (Table IV) No corresponding difference was found in women in whom the overall prevalence of a previous infarction was lower than in men. Angina pectoris more than 28 days before death was even slightly more common in than in men among those autopsied but -autopsied cases showed an opposite trend A y of a previous angina was more common in autopsied in the pathologic departments than in those autopsied medico-legally ($p < 05$). Of non-autopsied fatal cases 80% had a history of symptomatic heart disease before death. A new angina pectoris appearing within 28 days preceding death was rather uncommon in all groups It was slightly less common among fatal cases of AIHD than among all persons suffering from an acute attack of IHD during the study period

Persons who died had visited a doctor more often within 28 days preceding death and visits due to premonitory symptoms were slightly more common than among all registered cases of AIHD (Table V) All visits ($p < 001$) and visits due to premonitory symptoms ($p < 01$) were more common in persons autopsied in the pathologic departments than in those autopsied medico-legally The use of digitalis was clearly rarest in men autopsied medico-legally and most common in non-autopsied women and persons autopsied in the pathologic departments.

Table VI *Prevalence of a recent myocardial infarct in persons autopsied in the pathologic and forensic departments*

	Necrosis		Rupture		Probable		No infarct		Insuff. data	
	No	%	No.	%	No	%	No	%	No	%
Pathologic departments										
Males	62	47	10	8	33	25	19	15	7	5
Females	23	58	4	10	7	17	6	15	0	
Forensic departments										
Males	13	6	2	1	100	49	90	44	0	
Females	2	4	3	5	29	52	21	37	1	2
All autopsied deaths	100	23	19	4	169	39	124	32	8	2

Macroscopic discoloration in the myocardium and/or histologic lesions from a recent infarct.

Table VII. *Prevalence of an old inf rct scar the scar in a papillary muscle as the only old lesion and othe myocardial fibrosis in persons autopsied at the pathologic and forensic departments*

	Old infarct scar		Scar only in a papillary muscle		Other fibrosis		N old lesions		Insuff data
	No.	%	No.	%	No.	%	No.	%	No.
Pathologic departments									
Males	67	51	5	4	2	2	53	40	4
Females	19	48	3	7	0		18	45	0
Forensic departments									
Males	124	61	8	4	24	12	48	23	1
Females	24	43	4	7	7	12	21	38	0
All autopsied deaths	234	54	20	5	33	8	140	32	5

Autopsy findings

A necrotic myocardial infarction was found at autopsy in 58 % of the persons examined in the pathologic departments but in only 7 7 % of medicolegal autopsies (Table VI) Definite or probable evidence of an acute ischemic myocardial lesion was obtained in 81 % of the former cases. In medicolegally autopsied persons too an acute infarction was demonstrated in 57 % No clear difference in sex was found in this respect in either series A ruptured infarction was observed in 4.5 % of the present autopsied persons Rupture was more common in women (7.3 %) than in men (3 6 %) and more common in persons autopsied in the pathologic depart ments (8.2 %) than in those autopsied medicolegally (1.9 %). An infarct lesion in the anterior wall of the left ventricle was relatively more common in the former and a lesion in the posterior wall in the latter (Fig. 1) Of acute infarctions detected at medico-legal autopsies 81 % involved at least to some extent the interventricular septum while the corresponding frequency at autopsies in the pathologic departments was 53 % ($p <$ 001) The location of lesions was certainly more carefully recorded in the former than in the latter In medico-legal cases an acute infarction was found in the posterior wall of the right ventricle as an extension of a posterior infarction in 16 % of cases with a posterior lesion A papillary muscle was involved in 42.5 % of medico-legal autopsies. Of anterior infarctions 59 % extended to the papillary muscle and 52 % of posterior infarctions to the posterior papillary muscle.

An old myocardial infarction was found at autopsy more often in men (57 %) than in women (45 %) ($p <$.05) but with about a similar frequency in persons autopsied in the pathologic and forensic departments (Table VII) A similar situation was observed for the size of hearts heavier organs in men than in women but no difference between persons autopsied in different places (Table VIII). An aneurysm in a great scar of the left ventricular wall was found in 14 % of cases with an old infarction at autopsies in the pathologic departments and in 12 % at medico-legal autopsies The overall prevalence of an aneurysm was 7 % of all autopsies An

Table VIII *Heart weight in persons autopsied in the pathologic and forensic departments*

	Data available	Heart weight (g) Mean	S D.	Median	Range
Pathologic departments					
Males	116	532	126	520	40–990
Females	37	439	106	406	300–650
Forensic departments					
Males	195	517	121	490	320–1 020
Females	55	436	96	430	250–660

infarct scar was equally common in the anterior and posterior walls of the left ventricle (Fig 2) Of old infarctions in medico-legal cases 52 % and of lesions in persons autopsied in the pathologic departments 51 % were located at least to some extent in the myocardium of the interventricular septum A scar in a papillary muscle as the sole old lesion was found in 5 % of autopsied cases

A fresh coronary thrombosis was recorded in 29 % of autopsies from the pathologic departments and in 23 % of medico-legal autopsies The overall prevalence of an occlusion in any of the coronaries was 56 % in the former and 53 % in the latter the difference not being significant. The reported frequencies of an occlusion in different coronary arteries were as follows Autopsies in the pathologic departments. right coronary artery 32 % left anterior descending coronary artery 38 % and left circumflex coronary artery 19 % Medico-legal autopsies right coronary artery 32 % left anterior descending coronary artery 26 % and left circumflex coronary artery 18 % No significant difference in sex was found in relation to the prevalence of coronary occlusions (56 % for men 52 % for women) In 11 % of the cases no marked stenosis was observed in coronaries Such cases were reported more often from the pathologic (18 %) than from the forensic departments (7 %)

DISCUSSION

The reliability of the diagnosis of registered IHD deaths appeared to be good in the present community study which was based on a population of 550 000 inhabitants of Helsinki. For practical reasons it was not possible to apply a special methodology to the patho-anatomic investigation of hearts in all departments of pathology in the city Since the reliability of the diagnosis of IHD death certainly is most problematic in cases of sudden death special attention was focused according to the suggestions of the WHO (32) on the examination of medico-legal cases As the autopsy findings in other victims were based on the routine work performed by several pathologists the detailed comparisons with regard to the patho-anatomic findings may be regarded only as indicative of trends. Another problem in the retrospective study was caused by differences in the reliability of data of the disease history of the persons studied It was possible to get first-hand information directly from most patients dying in hospitals while in cases of sudden death occurring outside hospitals the information was obtained from relatives or other persons

Although the patho-anatomic signs of chronic heart disease the size of the heart and the occurrence of an old infarction were similar in persons autopsied in the pathologic and forensic departments a known symptomatic heart disease visits to a doctor and use of digitalis were clearly less common in the latter than in the former The discrepancy between the clinical history of a previous infarction and the autopsy finding of an old myocardial infarction was found in particular in medico-legally autopsied cases The second-hand nature of information on the disease history in cases of sudden death may in part, explain this discrepancy On the other hand myocardial changes in an acute infarction are visible by conventional macroscopic and histologic methods at autopsy after some 6–12 hours duration of ischemia (12 15) A recent detectable infarction was however rather common in the medico-legal autopsy material in persons whose fatal attack terminated rapidly nearly instantaneously or at least within one hour of its onset. It is probable that many patients who had died suddenly outside hospitals had refused to admit their symptoms failed to distinguish them from their usual complaints or interpreted them as originating from organs other than the heart.

A higher prevalence of acute myocardial infarction in persons autopsied in the pathologic departments than in those autopsied medico-legally fulfilled expectations The prevalence of infarct changes in the myocardium in the latter cases was very high compared with many previous series of sudden deaths in which the frequency has varied from 8 to 28 % (1 10 14 30) The present frequencies are also higher than that found in a community study in Rochester USA (27) where a recent myocardial infarction was found in 39 % of all autopsied IHD deaths A careful systematic examination of hearts by conventional methods also gives a rather high prevalence of infarct changes in sudden deaths An acute infarction was demonstrated at autopsy in 40 % of sudden deaths in the series of Titus et al (28) and in 47 % in the series of Scott and Briggs (24) The principles delineated in the WHO cooperative study (33) were followed in the investigation of the majority of the present medico-legal cases Since no generally agreed minimum criteria for the patho-anatomic diagnosis of an acute infarction exist, the

comparison of the findings made by different workers is difficult.

The anterior location of an acute infarction tended to be most common in persons autopsied in the pathologic departments and the posterior location in those autopsied medico-legally. The arterial disease, the highest frequency of an occlusion in the left anterior descending coronary artery in the former and in the right coronary artery in the latter, was in accordance with the distribution of infarct changes. Titus et al. (28) have reported a different distribution of infarctions in a series of sudden unexpected deaths, 53 % of lesions located in the anterior wall of the left ventricle and 36 % in the posterior wall. When sudden death outside hospitals was attributed to an acute infarction in the present series, the interventricular septum was involved in a high percentage of cases (81 %). On the other hand, in the same medico-legal series the interventricular septum was involved in only 52 % of cases with an old infarction, i.e. an infarction after which the persons had survived. The present observations suggest that the risk of sudden death is high in association with posterior infarctions and particularly with infarctions which involve the myocardium of the interventricular septum. Clinical studies have shown that dangerous bradyarrhythmic complications are common in the initial phase of an acute infarction, especially in association with inferior lesions (2, 9).

There was no difference in the size of the heart, the prevalence of an old infarction or the frequency of coronary occlusions between persons autopsied in the pathologic and forensic departments. The patho-anatomic condition of the heart before the fatal attack had probably not influenced the distribution of persons dying suddenly outside and those dying in hospitals. The prevalence of infarct scars in the present series was very similar to previous findings in a community study in Rochester (27), a series collected from several centres in Finland (18) and a Swedish series of medically unattended sudden deaths (30). A higher prevalence (69 %) of old or healing infarctions was reported in a series of deaths from an acute infarction by Scott and Briggs (24). A clinical history of a previous infarction has been reported in only about 30 % of persons dying suddenly (4, 6). A common finding in postmortem series is that the number of myocardial scars exceed that of clinically recognized episodes (21, 25, 30). Titus et al. (28) found a myocardial scar in 18 % of persons in whom sudden death was the first clinical manifestation of IHD. Clinical prospective studies have shown that an unrecognized infarction is rare in patients with previous angina pectoris but more likely in patients with previous diabetes or hypertension (16).

Among the autopsied persons in the present study a history of previous angina pectoris was about equally common in both sexes. Old myocardial infarctions were, however, clearly more common in men, particularly in the medico-legal series. Clinical prospective studies have shown that uncomplicated angina in a population is about as frequent in women as in men but IHD in women is clearly less often complicated by an attack of infarction (11). According to Lehn (13) the heart weight ratio in healthy men: healthy women is about 1.2 : 1. The mean values for heart weight in men and women in the present material were related in a similar way. Although the difference in the absolute heart size was marked between series, the cardiac enlargement had obviously occurred proportionally to the same extent in both sexes.

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ISOLATED VALVULAR AORTIC STENOSIS

Clinico-pathological Findings in an Autopsy Material of Elderly patients

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Abstract In a review of 2 557 autopsy reports from general hospital 20 patients, aged 65 or above, have been found to have severe isolated valvular aortic stenosis. The diagnosis was made or suggested clinically in only 5 cases. The clinical findings were often atypical, e.g. the BP amplitude was above 50 mmHg in 9 patients and 4 had systolic BP of 180 mmHg or more. Left ventricular hypertrophy as evaluated by ECG was however present in all examined cases but one The main cause of death was cardiac in 17 cases of whom 2 died suddenly and 4 following non-cardiac surgery At autopsy significant coronary arteriosclerosis was found in all but 2 patients. In 9 patients acute myocardial infarction was demonstrated—in cases only through a macroscopic enzyme test (Nitro-BT method).

In recent years the beds of medical departments have increasingly been used for treatment of elderly patients Although heart diseases in this age group are mainly of ischaemic etiology rarer causes of heart failure are naturally also met. We have recently noticed that a number of elderly heart patients at autopsy were found to have isolated valvular aortic stenosis (IVAS). The purpose of this paper is to describe the clinical and pathological findings in these cases.

MATERIAL AND METHOD

The material consists of 20 cases (9 men and 11 women) of IVAS aged 65 or above (range 65–89). The average age for men was 75.6, for women 77.6 years. The age and sex distribution appears in Table I. These cases were found by examining autopsy reports from the Institute of Pathology Sundby Hospital, during Jan 1968 – April 1972. Thi Institute serves population of approximately 120 000 persons. During this period 2 557 autopsies were performed. Only cases in which it as clearly indicated that the aortic ostium was narrowed and just allowed the passage of finger (grade 1) or less (grade 2) were included. All cases with changes localized to valves in mitral or tricuspidal ostium ere excluded.

Complete autopsy of the heart was performed in all cases. The heart was weighed and the outer dimensions were measured transversely along the sulcus coronarius and longitudinally from the latter to the apex cordis The ostia of the heart were explored and the sizes estimated The type of IVAS was evaluated according to the criteria of Pomerance (12) The coronary arteries and later the heart were cut open The thickness of the myocardium of the left and the right ventricle was measured. The myocardium was then cut and examined for acute myocardial infarction (AMI) and/or fibrosis. The extent of diffuse arteriosclerotic changes in the coronary arteries was graded (0–3). Localized, severe stenosing arterioscleroses as well as subintimal hemorrhages and thrombi were also recorded.

RESULTS

Clinical findings (Table I). Fourteen of 20 patients died in a medical department. Of the remaining 6 patients 4 were admitted to a surgical ward one to a gynecological ward and one to an ear nose and throat department. Two of the 20 patients died within 1 hour after admission, one during the first day 5 within the first week and 8 within 3 weeks and 4 21–30 days after admission.

Ten patients had had symptoms attributable to aortic disease for 1 year or longer 5 for less than / year Information about 5 patients was insufficient. Fifteen patients had dyspnea prior to hospitalization during which they died In 3 patients no dyspnea prior to admission was noted, information about 2 was insufficient. Ten patients had precordial pain prior to admission, 6 did not have In 4 cases it is not known whether they had precordial pain or not. Three patients had syncope In 7 it was not clear whether they had syncope or not. Aortic stenosis (AS) was diagnosed clinically in 4 cases and considered in 1 case In 15 cases the diagnosis of AS was not mentioned. Systolic murmur of typical location

In our experience LVH as seen in the ECG was present in 17 of 19 cases examined (1 of the 2 cases who did not show LVH had only standard leads taken). Although the presence of LVH in the ECG should give support to the suspicion of AS it should be remembered that it is difficult to know whether the established criteria for LVH also apply to the elderly and that other causes of LVH e.g hypertension are present particularly in the elderly

Useful X-ray examination was only made in a few cases as mentioned due to the patients clinical condition Enlargement of the left ventricle and dilatation of the ascending aorta are signs of aortic valvular stenosis These signs were also found in the majority of our cases In the elderly they are diagnostically less helpful as they are commonly seen in other diseases such as arterial hypertension and arteriosclerosis of the aorta and the coronary arteries.

This study does not allow any conclusion as to the prognosis of severe IVAS Thirteen of the 20 patients died however 2 of them suddenly directly as a consequence of their heart disease In 4 other cases who died following operation IVAS played a significant role. Thus in 17 of 20 cases IVAS was the major cause of death although a number of con- [illegible] factors existed Among the findings at au- [illegible] the most noteworthy is perhaps the extent of [illegible] osis in coronary arteries Only 2 of the 20 [illegible] ients did not have moderate to severe diffuse arteriosclerosis Almost occluding localized stenosis of the coronary arteries was, moreover found in 11 patients

These findings are in agreement with those of Roberts et al (13) In our material we thus find nothing to support the view that patients with IVAS often show very little arteriosclerosis of the coronary vessels nor do we find a basis for the theory that the degree of IVAS is inversely proportional to the degree of coronary arteriosclerosis as mentioned by Horan and Barnes (7) The incidence of AMI is high in our material (45%) Kumpe and Bean (8) found in a similar material an incidence of 12.1% Robert et al (13) 4% and Bergeron et al (3) only 3% As in the two latter reports we find that the myocardial infarctions were non-thrombotic two-thirds of the infarctions in the material of Kumpe and Bean (8) were of the same type These findings are in clear contrast to the previously accepted opinion that AMI is seldom seen in patients with IVAS (9)

The high incidence of myocardial infarction found in this study is partially due to the use of the Nitro-BT method (1) as myocardial infarction in 2 cases was not seen by ordinary gross examination but was demonstrated by the former method. As regards the pathogenesis of IVAS the distribution according to types in our material is in agreement with the findings of Pomerance (12). It is thus our conclusion that IVAS and death due to heart failure are common among elderly patients Severe and obstructing arteriosclerosis of the main coronary arteries is found in the majority of these patients. If valve surgery is considered in this age group, visualization of the coronary arteries should be contemplated since operative mortality among unselected elderly patients would be expected to be high.

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THE FREQUENCY OF SECONDARY HYPERTENSION

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Abstract In a series comprising 482 patients with hypertension requiring treatment 79% had to be classified as essential hypertension. Bilateral renal disease was found in 9%, unilateral renal disease in 3.3% but only one patient underwent surgery. Renal artery stenosis was found in 24 patients (5%), but only 5 (1%) were operated on. Two cases of primary hyperaldosteronism and one of phaeochromocytoma were found, in all three surgical intervention was successful. Oral contraceptives had caused the elevated BP in 7 patients (1.5%). It is emphasized that the frequency of curable hypertension is still very low and this should be taken into account when routine examination of patients with hypertension requiring treatment is discussed.

The majority of cases of elevated blood pressure must still be classified as essential hypertension also called primary hypertension In a certain group however the hypertension can be attributed to detectable conditions and is therefore termed secondary hypertension. The finding of such conditions is particularly important, since the underlying cause and thereby the hypertension may in some cases be remedied by surgical intervention. This is of course much to be preferred to prolonged drug therapy which in some cases is not well tolerated

Considering the high incidence of hypertension requiring treatment in the general population the study of hypertensive patients with the object of revealing specific etiological factors must be considered a rather demanding task and is consequently today a much discussed topic Such investigations must, of course be related to the incidence of secondary—including curable—hypertension. Therefore we should like to report on our experience in a group of hypertensive patients.

PATIENTS AND METHODS

The series includes 482 patients 272 males and 210 females, with elevated BP examined in our unit over the period 1958–72, with the object of revealing specific etiological factors The age distribution was as follows. 19% <40, 65% 40–59 and 16% >60 years of age.

Most of the patients were referred because of hypertension and, consequently the series must be considered to be selected The criterion for submitting the patients to an etiological study was that the hypertension was considered to require treatment along the lines established in Table I. The severity of the hypertension was assessed on the basis of the organic changes. Applying the Keith-Wagener classification of hypertensive retinopathy 69% had grades I and II 14% grade III without fresh exudates 12% grade III with fresh xudates and 5% had grade IV The serum creatinine level was normal in 72% 1.4–2.9 mg/100 ml in 25% and higher than 3.0 mg/100 ml in 3%.

Since the value of series which is intended to clarify the incidence of secondary hypertension depends very much on consistent programme of study the procedures will be described in detail.

Urography was performed in 464 patients (96%), i.e. not carried out in 18 patients. The reasons for omitting this investigation were as follows. two patients died suddenly 14 had severe uraemia, one patient was allergic to contrast media, and in one woman who became normotensive after having stopped taking oral contraceptives the examination was simply forgotten

During the first few years rapid sequence urograms were taken followed by tomograms. During recent years we have applied excretion urography As supplement we previously used renal angiography later isotope renography Angiography was performed in 32% of the cases and renography in 44%. These supplementary examinations were not made if the patient died shortly after admission or if pronounced uraemia was present. They were also omitted in patients with only one kidney and in very old or sick patients who could not tolerate surgery

Serum potassium was determined in all cases in 79% before commencement of treatment (A more de-

Table I. *Indications for antihypertensive therapy*

Organic changes	
Ocular	Hypertensive retinopathy grade IV Hypertensive retinopathy grade III with fresh exudates Hypertensive retinopathy grade II in younger patients
Cardial	Congestive heart failure Angina pectoris Past myocardial infarction Cardiac enlargement Pathological ECG
Cerebral	Acute encephalopathy Past strok (suitable cases)
Permanent diastolic hypertension at the age of	Diastolic BP (mmHg)
<40	≥100
40–49	≥105
≥50	≥110

tailed analysis of the potassium values found will be given later.)

During the first part of the study screening for phaeochromocytoma wa carried out by means of the regitine test, which was soon replaced by determination of catecholamines and/or examination of 24-hour urine specimens for vanillomandelic acid content. These tests were made in 378 patients (78%). In the remaining 104 p tients the tests were omitted in 38, in whom the diagnosis of phaeochromocytoma could be clearly excluded or would be of no clinical interest. During the retrospectiv tudy however we realized that in 66 patients the test should have been done routinely although in these cases there was no clinical evidence of phaeochromocytoma. (It will be shown later in this paper that routine examination did not reveal any case of phaeochromocytoma, the only case was found by X-ray.)

Finally we tried to palpate the femoral artery in all the patients—at least in those below the age of 40. Young females were questioned about their use of oral contraceptives. We have not made any systematic inquiry as to intake of liquorice.

Table II. *Distribution of patients according to etiology*

	N	%	Surgically treated
Essential hypertension	382	79.3	
Bilateral renal disease	45	9.3	
Unilateral renal disease	16	3.3	1
Renal artery stenosis	24	5.0	5
Special cases	5	1.0	
Primary hyperaldosteronism	2	0.4	2
Phaeochromocytoma	1	0.2	1
Oral contraceptives	7	1.5	
Total	482	100.0	9

Table III. *Incidence of renal diseases*

Diagnosis	
Bilateral renal disease	
Chronic glomerulonephritis	14
Chronic pyelonephritis	16
Chronic interstitial nephritis	9
Diabetic nephropathy	2
Polycystic kidneys	3
Medullary sponge kidney	1
Unilateral renal diseases	
Congenital aplastic kidney	6
Pyelonephritic contracted kidney	6
Hydronephrosis	2
Renal cyst	1
Renal dysplasia	1

RESULTS

The classification of the 482 patients is given in Table II. In 79% the hypertension had to be classified as essential.

Bilateral renal disease was found in 45 patients and unilateral renal disease in 16 (Table III). Only *one patient underwent surgery*, an 18-year-old female with congenital aplastic kidney, but the outcome was poor. All other patients received drug treatment.

Renal artery stenosis was found or strongly suspected in 24 patients (5%). In 9 cases we were, however, unable to confirm the finding of renovascular hypertension. Nine of the remaining patients received drug treatment, firstly because this proved easily manageable and effective (6 patients), and secondly because surgery was contraindicated (3 patients). Five patients underwent surgery: three became normotensive, one improved and in one patient the condition was unchanged. Finally one female with symptoms of fibromuscular hyperplasia is still under observation; her BP became normalized spontaneously.

In 5 patients (classified as special cases in Table II) a specific endocrine etiology was suspected. One patient with hypertension of moderate severity had a very high serum renin level and

Table IV *Evaluation of cas s with low serum potassium level*

	Untreated	Receiving diuretics
Response to potassium supplement	5	5
Response to spironolactone	1	3
Renin/aldosterone determination	3	1
Clinical course	6	5
Autopsy	2	6
Primary hyperaldosteronism	2	0
Other endocrine disorders possible	1	1
Total	20	21

aldosterone excretion without any demonstrable renal cause In three patients changes in the renin and aldosterone levels were found which were incompatible with primary hyperaldosteronism, but which might indicate the presence of another mineral corticoid metabolic disorder In all these cases the hypertension was adequately controlled by drug treatment. One patient had Turner s syndrome.

Two cases of primary hyperaldosteronism were found and in both the hypertension was relieved by surgery Since primary hyperaldosteronism must be suspected in any case of hypokalaemia, we shall describe the cases in which the serum potassium levels were below 3.5 mEq/l, i e 20 untreated patients and 21 patients who received diuretics at the commencement of the study With the object of further elucidating the cause we have evaluated the following features pronounced effect of potassium supplement, lack of effect of spironolactone treatment, determination of plasma renin and aldosterone production The remaining features were assessed on the basis of the clinical course or at autopsy (Table IV) Apart from the two cases described above primary hyperaldosteronism can in our opinion be excluded in the remaining patients

One case of phaeochromocytoma was found and operated on with favourable results. No cases of Cushing s syndrome were found

In 7 cases oral contraceptives were the cause of the hypertension the influence of such drugs being proved during several observation periods with and without oral contraceptives. In all cases the hypertension was of moderate severity only

Table V *Classification of the etiology of the hypertension in three series*

	Kennedy et al. (4)	Hood and Björk (2)	Present series
No. of pats.	750 (%)	685 (%)	482 (%)
Essential hypertension	72.6	39	79.3
Bilateral renal disease	7 7	8.9	9.3
Unilateral renal disease	9.5	1.9	3.3
Renal artery stenosis	6.1	13 7	5.0
Primary hyperaldosteronism	0.3	0.3	0.[illegible]
Cushing s syndrome	–	0.1	
Coarctation of aorta	–	1.0	–
Oral contraceptives	–	–	1.5
Other cases	–	24 4	1 0
Incompletely examined	3 7	9	–

In two younger patients the consumption of liquorice was excessive but the increase in BP was not remedied when the intake of liquorice was stopped. No cases of coarctation of the aorta were found.

DISCUSSION

A few surveys of the same nature as our study have been made. Our results and those from two earlier studies are summarized in Table V How ever it is extremely difficult to compare these series since both the diagnostic criteria and the selection of the patients differ Our results agree well with those found by Kennedy et al. (4). In the material presented by Hood and Björk (2) the incidence of essential hypertension is re markably low However the latter series comprised two groups, unclassifiable (24%) and incompletely investigated (9%) which probably included some cases of essential hypertension Furthermore, this material might well be specially selected in that several of the patients must be presumed to have been referred to the Gothenborg Centre because of special diagnostic problems. The age distribution in the series will also exert a certain influence because as is known from various surveys (1 7 9), the causes of the hypertension will often be specific in younger age groups Platt (8) found secondary hypertension in 70% of patients below the age of 35

Although it is reasonable to presume that a specific etiology might be detected or sus in about 20% of the cases when the seri

prises hospitalized patients with hypertension re quiring treatment, it does not follow that radical treatment will be possible in such cases

Bilateral renal disease is present in about half the cases of secondary hypertension (8–9%) and in these cases drug therapy is the treatment of choice In our material 14 patients had chronic glomerulonephritis which must be considered the cause of the hypertension. In the remaining patients of this group the etiological influence of the renal disease is doubtful since the concurrent appearance of essential hypertension and renal disease cannot be definitely excluded. This is particularly the case in patients with suspected chronic pyelonephritis.

The reported frequency of unilateral renal disease as a possible cause of the hypertension varies greatly (2–10%) and this is no doubt accounted for by the uncertainty with respect to the importance of this etiological factor In the 1940s a number of cases were reported in which surgery had had favourable effects but it appeared later that over a longer period the results were disappointing (10) a good deal of scepticism prevails on this point. In a series reported by Kirkendall et al (5) a permanent fall in BP was obtained in only one patient of 10 operated upon for unilateral renal disease

In 16 of our cases (3.5%) unilateral renal disease was considered to be the possible etiological factor but in only one case was surgery carried out and with a negative result. The remaining patients received drug treatment. However with the examination technique now available, in particular unilateral renal vein catheterization with renin determination, it will no doubt be possible to predict the result of surgical intervention more accurately When evaluating our series retrospectively we find that such examinations should have been done in at least 4 cases, although in two of them surgery was definitely contraindicated

Renal artery stenosis as the cause of hypertension has been of current interest during the last 10–15 years and is today the most frequent cause of surgically treatable hypertension. Good results have been obtained in many of the cases operated on, but the selection is quite decisive. Narrowing of the renal arteries found by angiography is rarely a sufficient indication for operation, such changes in the lumen are very frequent in hypertensive patients and also in normotensive persons, particularly after the age of 50 Consequently when selecting patients for surgery the decision should also be based on other examinations of which unilateral renin determination is the most important. However in many patients in particular the elderly drug treatment should be preferred

The incidence of renal artery stenosis in a series of hypertensive patients varies greatly e.g. depending on the value attached to the angiographic findings In our patients we have stated renal artery stenosis to be the possible cause in 24 (5%) but only 5 were operated on (1%) With the indications which are commonly followed today the frequency of operated renal artery stenosis would range between 1 and 2% in a group of hypertensive patients

We encountered two cases of primary hyperaldosteronism and one with phaeochromocytoma. The incidence of these rare diseases in our patients agrees well with that found in other series (Table V) It should be emphasized that no cases of phaeochromocytoma were revealed by the usual screening methods our only case was found by X-ray

Finally we had 7 cases in which withdrawal of oral contraceptives normalized the high BP As regards this problem reference is made to other reports (3 6).

As stated in the introduction, examination of patients with hypertension with a view to revealing any specific etiology is extremely important and some authors have maintained categorically that such extensive examinations should be performed in every case of hypertension requiring treatment. However such efforts should also be reasonably related to the available possibilities for radical treatment. In our series this was the case or had been attempted in 9 patients of 482 i.e. in 2%. Hence it must be realized that curable hypertension still represents a very limited proportion of our patients with hypertension.

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Table I. *Results of fine needle aspiration biopsy with two different staining techniques*

Pat. no	Sex.	Age (y)	Palpable neck abnormality	May–Grünwald–Giemsa: Malignant C-cells	May–Grünwald–Giemsa: Normal cytology	Alkaline Congo amyloid positive
Group A						
1	♀	32	+	+		+
2	♂	57	+	+		+
3	♀	22	+	+		
4	♀	54	Absent		+	
5	♂	58	+	+		+
6	♂	59	Absent	Biopsy material insufficient for evaluation		
7	♂	33	+	+		+
8	♀	35	+		+	
9	♀	22	Absent	Biopsy material insufficient for evaluation		
10	♂	22	Absent	+		+
Group B						
11	♀	72	+	+		Not examined
12	♀	77	+	+		+
13	♀	36	+	+		
14	♂	71	+	+		+
15	♂	51	+	+		+
16	♂	46	+	+		+
Group C						
17	♂	72	+	+		
18	♂	54	Absent	Biopsy material insufficient for evaluation		
Total			13/18	13/15	2/15	9/14

Hazard et al. (7) and Williams et al. (25). The other 2 were ly proved carriers of the Sipple genome, had high levels of S-CT as well as pheochromo- but refused surgery for MCT (nos. 6 and 8). patients are divided into three groups A B and C (Table I)

Group A New cases of hereditary MCT Three families with Sipple's syndrome (22), altogether 47 members were screened prospectively for MCT and pheochromocytoma. This screening program includes 1) careful clinical examination, 2) thyroid scintiscan using ^{131}I and/or ^{99m}Tc 3) determination of S-CT both (*a*) basal level and (*b*) the response to induced hypercalcaemia, using our radioimmunoassay method for human S-CT (1), 4) determination of diamine oxidase activity (E.C.1 4.3.6.histaminase) according to Tufvesson and Tryding (23), and 5) fine needle aspiration biopsy of all palpable thyroid nodules or lymph nodes of the neck ("palpable neck abnormality" in Table I) (In 4 patients (nos. 4 6, 9 10) aspiration biopsy from the thyroid was attempted although there were no palpable neck abnormalities.) In these families 10 new patients with subsequently verified MCT were found (nos 1 10).

Group B New sporadic case (seen in the Outpatient Clinic for thyroid disorders). Six patients without known heredity for Sipple's syndrome were examined because of nodular goitre (nos. 11–13) or neck lymphoglandular metastases from primary tumours, the nature and localization of which was unknown (nos. 14–16)

Group C Cases of earlier operated sporadic MCT Two patients were previously hemithyreoidectomized for sporadic MCT One presented with large mass in the neck (no 17), the other had no palpable abnormality but raised S-CT and microscopically there was insufficient radicality of the surgical specimen (no. 18). Fine needle biopsy was performed from beneath the scar of the previous thyroid operation These patients were examined by us to verify suspected metastases of MCT

Controls To study the specificity of amyloid in MCT we examined 60 fine needle aspiration biopsies from 30 consecutive patients with miscellaneous types of goitre other than MCT seen as outpatients in our clinic for thyroid disorders. Smears from each patient were stained with May–Grünwald–Giemsa and with alkaline Congo.

METHODS

The instruments used for the aspiration biopsies were either the one-hand syringe of Franzén et al (6) or disposable plastic syringes (10 ml) with an adaptor (Cameco, Sweden). Both are so constructed that an excellent grip for one-handed manipulation during the bioptic procedure is possible. The *puncture technique* has been thoroughly described previously (19). During maximum aspiration the needle is moved back and forth in the puncture channel for few seconds. Prolonged aspiration often causes too heavy an admixture of blood Care must be taken to discontinue the aspiration before the needle is withdrawn, otherwise the aspirate will spread on the walls of the syringe



Fig 1 MCT cells as they appear in wet-fixed aspirates stained with hematoxylin-eosin, 400.



Fig 4 Some aspirate specimens of MCT present monomorphous and non-specific populations of small tumour cells in which the diagnostic red-granulated cells (giant cell in centre) have to be sought for. May-Grünwald-Giemsa, 400.

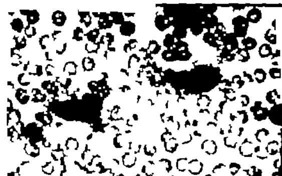


Fig 2 Two MCT cells with the angulated, "dendritic cell" appearance which is common in some tumours. May-Grünwald-Giemsa, 400.



Fig 5 MCT cells as they appear in dry fixed aspirates stained with May-Grünwald-Giemsa. Note the single multinucleated red-granulated cell and the nuclei seemingly "about-to-leave" the cytoplasm in some of the small cells, feature rather typical of these APUD tumour cells in smears, 400.



Fig 3 The triangular cell in the centre is typical red-granulated MCT cell, cell to the left below presents the bluish-grey cytoplasmic condensations, thought to be intracellular amyloid. May-Grünwald-Giemsa, 1 000.

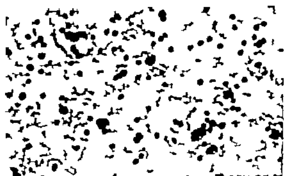


Fig 6 Survey with many cells characteristic of MCT. May-Grünwald-Giemsa, 100

All magnification figures represent the microscope magnification.

Table

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Fig 7. *Aspirate from MCT stained with Congo red for* amyloid viewed in non-polarized light to the right and with crossed polaroid filters to the left. A large amyloid deposit in the centre, 100.



Fig 10. Askanazy cells from a Hurthle cell adenoma, distinguished from MCT cells by their dusty blue granulation. May–Grünwald–Giemsa, 1 000.

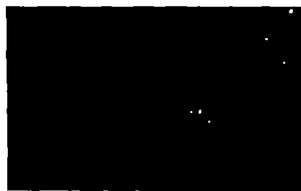


Fig 8. Smears should be screened under crossed polarization filters for the presence of the green luminescence of amyloid. Congo red, 100.



Fig 11. MCT cells (left) and pheochromocytoma cells (right) in smears from patient 11. May–Grünwald–Giemsa stained aspirates these tumours yield virtually identical cytological pictures, 400.

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gr



Fig 9. T[illegible]olet staining lumps of amorphous substance *thought to be amyloid. May–Grünwald–Giemsa, 1 000.*



Fig 12. Chemodectoma cells difficult to distinguish from *MCT cells. May–Grünwald–Giemsa, 1 000.*

All magnification figures represent the microscope magnification

and will be difficult to expel. The aspirate is carefully smeared out on one or more glass slides, the specimens for staining according to May–Grünwald–Giemsa being air dried, the others immediately fixed in 80% alcohol for staining ith hematoxylin-eosin and alkaline Congo (17). Some smears were stained with toluidine blue and eosin alone. It is possible to destain old slides with acid methanol and restain with alkaline Congo, which was done in our first cases of hereditary MCT and in the sporadic cases where the diagnosis of MCT was not primarily suspected. I these slides as well the amyloid deposits showed the typical green birefringence in polarized light. Some patients were biopsied several times, others only once.

Histological sections from the tumours were stained with hematoxylin-eosin, van Gieson and lkaline Congo

RESULTS

Characteristic morphology of MCT cells (May–Grünwald–Giemsa staining)

Cell size and shape. Most tumour cells appeared larger than the normal thyroid follicular cells, but all sizes were represented up to multinuclear giant cells (Figs. 1 4). Quite often the cells were spindleshaped or triangular with dendritic extensions. This cell shape was very characteristic and of high diagnostic significance which can be seen also in histological sections, as was pointed out by Tateishi et al. (20) (Fig. 2).

Cytoplasm. The rather abundant cytoplasm appeared amphophilic and structureless in most cells but in about 5–15% of the total cell population a distinct *red granul tion* was seen (Figs. ., 3). The granules were usually rather coarse but sometimes fine and dustlike. In a few cells the granulation appeared as a diffuse red staining in part of the cytoplasm.

The granules did not take stain with eosin alone and showed weak metachromasia w th toluid ne blue. In the terminology of MGG staining they may thus be defined as *azurophilic* (cf. the primary granules of promyelocytes) and not as eosinophilic. This explains why this type of granulation is not distinct in smears stained with hematoxylin-eosin, in which this eye-catching detail of the MCT cytology is lost.

The granulated cells had many specific features in common with the other cells of the population. They never appeared as a fundamentally separate type of cells but rather as differentiation extremes withi the variation spectrum of MCT cells to be compared e.g. with the fraction of melanin-granulated cells i fine needle samples from malignant melanomas.

In some carcinomas the granulated cell were rare and the smears consisted mainly of non-specific tumour cell with ver . . . little cytoplasm (Fig 4 pat ent 10

Nucleus. A most chara teri tic high proportion of binucleate cell In multinucleate cells as well were freq t were nearly always eccentrically p u cell and were dark and sharply out ne l Large and distinct nucleoli wer Nuclear pleomorphism was not marked giant multinuclear cells Mitoses were

In most aspirates the tumour cell we isolated and in groups the most b arr e ever often being isolated It was n t d distinguish the so-called Askanazy or H th from the MCT tumour cells The A k n ha e a dark-blue dustlike granulation stricted to part of the "smoke-gre (Fig 10) (16)

Amyloid. In many smears ther wer amorphous lumps of a substance tain gr or violet with May–Grünwald–Giemsa h red with hematoxylin-eosin U ually th t was located outside the cells but s r seemed to be present also intracellularly (F 9). With alkaline Congo the amyloid w light common light microscopy (Fig. 7). In polarized light the amyloid showed the characteri tic gree birefringence (Figs 7 8). Material sufficient for diagnostic evaluation with alkaline Congo stain was obtained in 14 patients. 9 of them were pos ti e for amyloid (Table I). In none of our controls could we find extra or intracellular lumps of amorphous substance with amyloid criteria. Therefore we conclude that MCT amyloid does represent amyloid and not only denatured thyreoglobulin.

To summarize the whole tumour cell population looked rather alien in normal thyroid cell sampl from an aspiration biopsy and appeared clearly neoplastic (Fig. 6). The characteristic cell was triangular with one or more nuclei eccentrically placed. The rather abundant cytoplasm had a bright red granulation and between the cells and cell groups some times also intracellularly there were lumps of amyloid

Histology

All slides from the operative specimens were reviewed, specially with regard to the occurrence of amyloid deposits.

In all but one patient (no. 10) ther were varying

amounts of amyloid This patient had multiple tumours of peanut size in both lobes. The tumours were composed of uniform cells with rounded nuclei and rather scanty cytoplasm. The cells were arranged in trabeculae or in small follicular structures The tumour stroma was scanty and no amyloid could be seen in the histological sections Serial sectioning was not performed *Fine needle aspirates* from this patient, however showed a few minute deposits of amyloid. The smears were dominated by small non-specific tumour cells with only few single larger *red-granulated* cells (Fig. 4) Thus in this case cytology was more informative than histology The MCT diagnosis was further confirmed by the raised basal S-CT and the elevated response to induced hypercalcemia.

Diagnostic results

Material sufficient for diagnostic evaluation was obtained in 15 patients whereas the cell yield was insufficient for diagnosis in 3 (Table I). In these three patients (nos. 6 9 and 18) there were no palpable abnormalities to guide the puncture needle.

Correct diagnosis was established in 13 (87%) of the 15 patients with adequate biopsies *It should be* noted that one of these was the result of a "blind" puncture of the thyroid region without a convincing alpatory target (patient 10). Thus 2 of 15 patients e wrongly diagnosed However in one of these patients (no. 4) the tumour was so small that the id was considered normal by two clinicians perienced in thyroid disorders The other of them (no 8) had a soft nodular goitre in which the tumour obviously was hiding. *In* this patient the diagnosis has not been verified by surgery and histopathology but she belongs to a Sipple family and has been operated upon for pheochromocytoma thus being a certified Sipple gene carrier Repeated controls of S-CT have shown a successive rise from normal to definitely pathological levels Therefore we believe this patient to have multiple small MCT foci which have been missed by the needle

DISCUSSION

An early and specific diagnosis of MCT is of considerable clinical consequence This tumour is probably more common than is usually thought but its growth is sometimes slow and may therefore conceal the neoplastic nature of the condition The combination of MCT with bilateral pheochromocytomas in Sipple s syndrome may cause unexpected cardiovascular emergency situations when general anesthesia and operation are attempted This augments the need for an easy and reliable method of preoperative diagnosis of this tumour

At present we are trying several methods for this purpose In this paper the attention is drawn to the rather specific cytological morphology of MCT in fine needle aspirates.

Like other authors in Scandinavia (5 16) we have long had the attitude that no examination of a thyroid lesion is complete without a fine needle biopsy The subsequent evaluation of the results (27) has shown this policy to be justified even if the cytological diagnosis of follicular thyroid carcinoma is very difficult (19)

The diagnostic problem as to MCT is different. This tumour yields a cytology with characteristic features which usually permit the specific diagnosis a somewhat *asymmetrical tumour cell* with one or more nuclei eccentrically positioned, many of them having a distinct bright red granulation. The *amyloid deposits* with a green birefringence in polarized light—also well visible in May–Grünwald–Giemsa and hematoxylin-eosin staining—may finally secure the specific diagnosis.

Once the specific morphology of MCT cells had been recognized, similar cells interpreted as C-cells were detected in non-neoplastic thyroid tissue especially in thyrotoxicosis (12) However the few and isolated C-cells seen in such conditions never presented a problem of differential diagnosis in relation to MCT

Why do some or the majority of the MCT tumour cells appear without the characteristic granulation? In several other amine-containing cells with endocrine activities mast cells being the best studied, it has been shown that the granules are probably composed of a glycoseaminoglycan matrix the ion exchange properties of which retain both the amine and the often basic granule protein (3) Probably the neoplastic change of the cells impairs the storage capacity Thus in mastocytosis the metachromatic granules are fewer smaller and stain less intensely than usual (7) In all these cells we do not always know which one of the different substances accounts for the characteristic staining properties

The varying frequency of granulated cells in MCT also corresponds well with the finding that immunofluorescence technique could only detect immunoreactive calcitonin in 40% of the cells in a case of MCT (4)

Even if only a minority of the MCT cells show the characteristic red granulation these are of great diagnostic importance (26). However it is of practical and theoretical interest to note that fine needle aspirates of other neuroectodermal and APUD-cell tumours also exhibit red granulated cells that are akin to those of MCT pheochromocytomas (Fig. 11) chemodectomas (Fig. 12) and carcinoid tumours. This resemblance may evoke differential diagnostic problems when the biopsy specimen is taken from a mediastinal or neck tumour Sometimes mammary carcinomas and hypernephromas ha e red granulated cells though seldom or never creat ing a practical problem (own unpublished observa tions).

As the red granules of MCT are stained with May–Grünwald–G emsa but not with hematoxylin-eosin we conclude that May–Grünwald–Giemsa should always be used as a routine staining technique for thyroid aspirates

We also conclude from the present observations that the cloudy deposits seen in our MCT specimens consist of amyloid As such deposits seem to be present also within the cytoplasm of the tumour cells, it appears probable that this amyloid is produced within the tumour cell This opinion is shared by other authors (8 ‿5) The nature of the amyloid of MCT has not been elucidated

Amyloid was found in only 9/14 (64%) of our patients However it is well known that the distribution of these deposits is uneven and patchy and some tumours contain only little amyloid Thus it is not surprising that the yield of amyloid is rather hazardous in these aspirates The fact that some biopsies were stained with alkaline Congo only after destaining of air-dried-fixed May–Grünwald–Giemsa smears may also have contributed negatively to the results.

The di gnostic results of fine eedle aspiration biopsies as a whole may vary due to several factors If the patient has a *palpable abnormality* and/or an abnormal scintiscan to guide the needle the chance of getting a representative cell yield s very good. The diagnosis should not be missed provided that the technical treatment of the smear optimal. On the other hand to find the malignant MCT cells in patients with only small *non-palpabl tumour foci* may certainly be difficult, e g. as in the very early hereditary cases found in screening programs nd in already surgically treated patients However this method should be tried even in these cases as t offers a chance to get a positi e diagnosi with negligible discomfort and risk for the patient

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THE THYROID IN ULCERATIVE COLITIS AND CROHN'S DISEASE

I Thyroid Radioiodide Uptake and Urinary Iodine Excretion

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Abstract. In order to investigate the prevalence of iodine depletion in chronic inflammatory bowel disease two separate studies have been performed. One was devoted to the 24-hour urinary iodine excretion and 50 patients with ulcerative colitis or Crohn's disease were examined and compared with 102 controls. In the other study the thyroid ^{131}I uptake was compared in 38 patients and 36 controls. Ten of the 50 patients with chronic inflammatory bowel disease had a 24-hour urinary iodine excretion less than 40 μg, compared with 5 of the 102 controls ($p<0.01$). Sixteen of the 38 patients had a 24-hour thyroid ^{131}I uptake of 50% or more of the administered test dose, compared with 4 of the 36 controls ($p<0.01$). These results are compatible with an increased occurrence of iodine deficiency in patients with chronic inflammatory bowel disease. Treatment with corticosteroids or Salazopyrin or a milk-free diet did not influence these findings. No evidence was found of an impaired absorption of inorganic iodide from the gut.

Iodide is absorbed mainly from the small bowel (17). It then becomes part of the iodide pool from which it is taken up by the thyroid for production of thyroxine and triiodothyronine. The thyroid hormones are distributed to the tissues where they are deiodinated. Part of the liberated iodide is used by the thyroid again and part is excreted, the chief excretory routes being the urine and the faeces.

An earlier study raised the possibility of iodine deficiency in chronic inflammatory bowel disease (5). The aim of the present study was to compare the prevalence of iodine depletion in patients with chronic inflammatory bowel disease and healthy controls.

MATERIAL AND METHODS

Two separate studies were undertaken, in each of which the patients were asked to complete a questionnaire about their dietary habits with special regard to the consumption of sea fish, milk and the use of iodized table salt in cooking. The laboratory analyses were made in the Department of Clinical Chemistry, Linköping.

In the first study 24-hour urine samples were collected from a group of patients with inflammatory gut disease comprising 25 men and 25 women with the mean age of 29 years (range 13–57). They were analysed for iodine excretion by the Technicon PBI AutoAnalyzer (Technicon Instruments Ltd) without pretreatment with an anion exchange resin. Ten of the 50 patients had distal ulcerative colitis and 10 had extensive or universal disease. Twenty-eight of the patients had Crohn's disease: four of them had mainly anorectal lesions, 11 had more widespread colonic lesions and in 13 the small bowel was chiefly affected. In 2 patients the colitis was unclassified.

Each patient had a sigmoidoscopy and rectal biopsy performed together with radiologic examination of the colon and small bowel. Depending on the results of these studies and the clinical history a diagnosis of ulcerative colitis (UC) or Crohn's disease was made. A differential diagnosis could not be made in two of the cases. During the time of the study 15 patients were treated with corticosteroids and 32 with Salazopyrin. Forty patients, of whom 15 were on a milk-free diet, answered the dietary questionnaire. This group was compared with 102 controls (38 men, 64 women) without clinical evidence of thyroid or gastrointestinal disease. The mean age was 30.8 years (range 13–90).

In the second study the thyroid radioiodide uptake level was measured 24 hours after an oral test dose of 10 μCi ^{131}I, as potassium iodide, in a separate group of 20 males and 18 females with chronic inflammatory gut disease. The mean age of the patients was 29.[illegible] years (range 16–57). The *measurements were made according to the recommendations* of the IAEA consultants group (14). In each case the urinary radioiodine excretion was determined from a 24-hour sample following the test dose. In 35 of these 38 patients the 24-hour urinary iodine excretion was also measured within a few days before or after the ^{131}I study. *These 35 patients are included in the group of 50 patients in* the first study. Five of the 38 patients had distal and nine extensive or universal ulcerative colitis. Twenty-three patients had Crohn's disease: three of them had mainly anorectal changes, nine of the others had more widespread

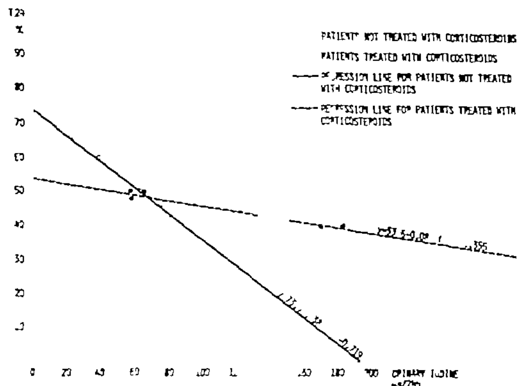


Fig 1 Correlation between the 24-hour thyroid [131]I uptake (*T 24*) and the 24-hour urinary iodine excretion in 35 patients with *inflammatory bowel disease*

our urinary iodine excretion of 40 μg or less is a rte indication of iodine deficiency and an excre- 40–70 μg is suggestive of incompletely filled ie stores

Corticosteroids diminish the thyroid accumulation of radioiodide and increase its urinary excretion (4 9 13). These effects are dose-dependent (1 11) In the present study no significant differences were found between patients treated with corticosteroids and those who did not receive such treatment. However if the correlation between the 24-hour thyroid [131]I uptake and the daily urinary iodine excretion is considered it appears as if corticosteroids do influence these variables and one of them more than the other As patients with more severe disease are more likely to be treated with corticosteroids it is possible that they in fact had a more pronounced iodine depletion than the other patients.

Salicylate which is a constituent of Salazopyrin® alters the protein binding of thyroxine (13 22) and increases its turnover (21) Hence it could have influenced the results. However after ingestion of Salazopyrin® in therapeutic dosage the serum concentrations of 5-aminosalicylic and acetyl-5-aminosalicylic acids are very low (16). In the present study no significant differences were found between patients treated with Salazopyrin® and those who did not receive such treatment.

The dietary intake of iodine has a great influence on observations of the type made in the present study The most important iodine sources are in foods such as sea fish, milk and iodized salt. In the present study the actual amounts of iodine ingested were not measured, but the consumption of sea fish was the same for patients and controls and all used iodized salt for cooking. Some of the patients were on a milk-free diet, but this did not significantly affect either their urinary iodine excretion or thyroid radioiodide uptake as compared with those patients who were on a non-restricted diet.

The mechanism of iodine depletion does not appear to depend on a reduced absorption of inorganic iodine from the inflamed gut, as the same proportion of the [131]I tracer dose could be detected in the urine and thyroid in patients and controls. Excessive loss of protein into the gut lumen is a well known complication of both UC and Crohn s disease As thyroxine is protein-bound it would be tempting to explain the increased prevalence of iodine depletion in these diseases as the result of a protein-losing entero-

pathy In fact a recent study has shown that thyroxine has an increased daily fractional turnover in UC and Crohn's disease (7) The thyroxine pool was very small in some patients. In some of the severely ill patients the daily disposal of thyroxine iodine was excessive and it is likely that, if the disease is of long duration this could be a cause of iodine depletion in patients with UC or Crohn's disease

In chronic inflammatory bowel disease a certain amount of bleeding often occurs even in cases in which no blood has been observed macroscopically in the stools (18) which could contribute to the iodine losses. In that respect it is interesting to note that the male patients had results almost identical with those of the female controls of whom most were in the fertile ages and had regular menstruations.

The practical implications of these findings are not yet fully evaluated but it may be relevant that a recent study in Oxford (6) has shown a significantly higher frequency of simple goitre in patients with ulcerative colitis than in controls matched for age and sex.

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Table I *Brief details of the patients and controls with a history of hyperthyroidism*

Case no	Present age (y)	Symptoms at the time of diagnosis	Involvement of colon	Time of occurrence of hyperthyroidism (y) Before UC	After UC
Among 300 patients with ulcerative colitis					
1	46	Hyperthyroid symptoms. Operated on. Histology: thyrotoxicosis	Extensive colitis	16	
2	47	Tremor Myopathy Thyroid bruit Thyroid tests and radioiodide uptake test within hyperthyroid range Treated with antithyroid drugs	Universal colitis		10
3	50	Tremor Sweating. Palpitations. Nervous. Eye symptoms. Thyroid radioiodide uptake test within hyper thyroid range Treated with antithyroid drugs	Distal colitis	8	
4	57	Tremor Sweating. Eye symptoms. Thyroid test and radioiodide uptake test within hyperthyroid range Treated with radioiodide	Universal colitis	1	
5	58	Weight loss. Eye symptoms Thyroid tests and radioiodide uptake test within hyperthyroid range Treated with radioiodide	Distal colitis	3	
6	59	Hyperthyroid symptoms. Operated on Histology: thyrotoxic goitre	Distal colitis		12
7	60	Weight loss. Unilateral exophthalmos Thyroid tests within hyperthyroid range Treated with antithyroid drugs	Universal colitis		19
8	66	Nervous Exophthalmos. Operated on Histology: thyrotoxicosis	Distal colitis	41	
9	70	Tremor Nervous. Exophthalmos Sweating Radioiodide uptake test within hyperthyroid range	Universal colitis	12	
10	73	Weight loss. Eye symptoms. Myopathy Thyroid test and radioiodide uptake test within hyperthyroid range. Treated with radioiodide and later antithyroid drugs after recurrence	Distal colitis	0*	0*
1	78	Hyperthyroid symptoms Operated on. Histology: thyrotoxicosis. Later pernicious anaemia	Distal colitis	9	
g 600 controls					
	38	Exophthalmos Sweating. Weight loss Nervous Thyroid tests within hyperthyroid range. Treated with antithyroid drugs			
2	44	Exophthalmos. Weight loss Thyroid tests within hyperthyroid range Treated with antithyroid drugs			
3	47	Exophthalmos. Weight loss Thyroid tests and radioiodide uptake test within hyperthyroid range Treated with antithyroid drugs			
4	56	Sweating. Palpitations Weight loss. Treated with antithyroid drugs Radioiodide treatment after recurrence			
5	59	Exophthalmos. Nervous. Weight loss Operated on. Hospital notes lost			

* The diagnoses of thyrotoxicosis and UC were made at the same time

cion of myxoedema that could not be confirmed On the contrary her thyroid activity was not suppressible with triiodothyronine and such a finding is very rare in the absence of hyperthyroidism or a nodular goitre (14) which she did not have However as she never had had thyrotoxic symptoms clinically she was not considered to be a case of hyperthyroidism The frequency of a firm history of hyperthyroidism in UC patients was 3 7% (Table II). No difference was found between patients with distal colitis and those with more substantial involvement of the colon in the liability to have a history of hyperthyroidism.

Among the controls five women gave a history of hyperthyroidism and had been treated for it The details of these women are given in Table I The frequency was 0.8% (Table II) i e significantly lower than in the UC group

No cases of hypothyroidism were found in the UC

Table II *Frequency of hyperthyroidism in ulcerative colitis patients compared with the frequency in controls*

	N	Hyperthyroidism		Significance of the difference
			%	
UC	300	11	3 7	X^2=9 19 =1
Controls	600	5	0 8	p<0.01

group In the control group two men and two women were being treated for this disease. The diagnosis in two of these patients could be definitely confirmed from the notes. No cases of thyroiditis or malignant thyroid disease were observed

One UC patient and five controls had been operated on for non-toxic goitres In evaluating the number of observed thyroid enlargements in these two groups these postoperative cases were regarded as examples of enlarged thyroids even if the thyroid was of normal size at the time of the study Table III shows the number of visible or palpable goitres found by the two observers As expected there were differences between the findings of the two observers but they both found a significantly higher frequency of enlarged thyroids in the UC group than in the control group.

Enlarged thyroids were noted with the same frequency among patients with distal colitis as among those with more widespread disease of the colon (Table IV) Likewise the duration of UC did not have any influence on the liability to an enlarged thyroid gland (Table V)

DISCUSSION

A study of this kind has two possible sources of error One is whether the control group is representative of the population. Patients admitted to the medical wards come from all parts of the Oxford area. They have varying occupations and represent all social classes Their visitors would have the same characteristics and therefore constitute a random sample of the population in the Oxford area.

The other difficulty is the interindividual disagreement on the size of the thyroid in borderline cases For the individual observer the only reference point is his own experience of what is normal or abnormal Thus observations are strictly comparable only when made by one and the same observer As expected there were differences between the findings of the two observers However the important point is that independently they found a significantly higher frequency of enlarged thyroids in the UC group than in the control group

It is common knowledge that simple goitre is usually associated with iodine deficiency The findings of a raised thyroid radioiodide uptake and reduced urinary iodine excretion in patients with ulcerative colitis are consistent with the occurrence of an increased prevalence of iodine depletion in this disease (13). However it is not known whether these abnormalities of iodine metabolism are long standing or simply occur for brief periods during and soon after acute attacks of this disease. If the chance of developing a simple goitre is increased by iodine loss through the inflamed colonic mucosa in UC one might expect that patients with extensive o um er sal colitis would show a higher prevalence of simple goitre than patients with distal UC and likewise patients with a long history of UC would show a higher prevalence of goitre than patients w th a short history Neither of these expectations has bee supported by the find ngs of the present tudy The explanation for the increased prevalence of simple goitre in subjects with UC remains uncertain

The frequency of hyperthyroidism in UC patients found in the present study is the highest reported so far This may be because all the patients and control were interviewed specifically about thyroid problems whereas routine case histories may be defective regarding the past history of the patients

It is worth noting that as shown n Table I a majority of the UC patients with a history of hyperthyroidism had suffered from the hyperthyroidism before they developed the col tis In other words the time of occurrence does not

Table III *Frequency of definite thyroid enlargement in ulcerative olitis patients and in controls*

	N	Enlarged thyroids		Significance of the difference
			%	
Observer 1				
UC	300	26	8.7	X^2=6 899 =1
Controls	600	26	4 3	p<0 01
Observer 2				
UC	300	19	6 3	X^2=4 342 =1
Controls	600	20	3.3	p<0 05

Table IV *Frequency of enlarged thyroid gland in ulcerative colitis in relation to the maximum extent of the colitis as judged radiologically*

The extent of the disease could not be decided in 5 subjects. Two of them were found to have enlarged thyroids by observer 1 and one by observer 2

Involvement of colon	N	Enlarged thyroids n	%	Significance of the difference
Observer 1				
Universal or extensive colitis	122	11	9 0	$X^2=0.216$ $n=1$ $p>0.05$ N.S
Distal colitis	173	13	7.5	
Observer 2				
Universal or extensive colitis	122	7	5 7	$X^2=0.048$ $=1$ $p>0\ 05$ N.S
Distal colitis	173	11	6.4	

support the view that hyperthyroidism is a complication of the bowel disease

It is widely believed that immunological disturbances participate in the pathogenesis of hyperthyroidism (5 19 22) Hyperthyroidism is known to be associated with some other diseases thought to be immunologically induced such as ...a gravis (8 18 21) Addison s disease (2 15) and pernicious anemia (20 25 28) A similar ...ship is also illustrated by the association of Addison s disease with pernicious anemia (1 2) There is also a strong association between myxoedema and pernicious anemia (27 28)

The thyroid gland and the stomach both derive from the primitive foregut and both have the ability to trap iodide (10 17) It is therefore not surprising to find a high incidence of thyroid antibodies in pernicious anemia (4) and conversely a high incidence of antigastric antibodies in hyperthyroidism (29). In Addison s disease there is also a great proportion of patients with thyroid antibodies (2)

The colon does not have the same embryological origin as the thyroid gland nor does it have the ability to concentrate iodide Thyroid antibodies occur in UC with the same frequency as in the normal population (17 26 30) In one study antigastric antibodies were found more frequently in UC patients than in controls but the difference was not statistically significant (30) while in another study these antibodies occurred with a similar frequency in UC patients and controls (17) Adrenal antibodies do not occur with undue frequency in UC (17)

Consequently it is more difficult to explain an association between thyroid disease and ulcerative colitis on an immunological basis than the association between thyroid disease and pernicious anemia. The isolation of a thyroid-stimulating factor from Clostridium perfringens (16) may give a clue to the relationship between hyperthyroidism and chronic inflammatory bowel disease However this factor needs to be further studied before any conclusions can be drawn. It is common clinical experience that both hyperthyroidism and acute attacks of UC often seem to be initiated by psychological trauma in the setting of emotionally stressful situations It is therefore tempting to ascribe the association between these two conditions to psychosomatic factors which could initiate hyperthyroidism in some subjects UC in others and both these diseases in a few However McKenzie (19) has said of hyperthyroidism that "despite a vast literature on the subject there is no convincing evidence of how or if a psychological or emotional determinant is concerned in the apparently complex pathogenesis and the same is certainly true of ulcerative colitis

Table V *Frequency of enlarged thyroid gland in ulcerative colitis patients in relation to duration of the colitis*

Duration (y)	N	Enlarged thyroids n	%	Significance of the difference
Observer 1				
<8	155	12	7 7	$X^2=0.346$ $=1$ $p>0\ 05$ N.S
>8	145	14	9 7	
Observer 2				
<8	155	6	3 9	$X^2=3.278$ $=1$ $p>0\ 05$ N S
>8	145	13	9 0	

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THE THYROID IN ULCERATIVE COLITIS AND CROHN S DISEASE

III *The Daily Fractional Turnover of Thyroxine*

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Abstract The daily fractional turnover of thyroxine (T_4) labelled with ^{125}I has been determined in 11 patients with ulcerative colitis (UC) or Crohn disease and 8 controls. The daily fractional turnover of ^{125}I T was significantly increased in the patient group. The daily total disposal of T iodine was not significantly different although it was excessive in 3 of the 11 patients. The amount of T in plasma did not differ significantly between the patients and the controls. It is concluded that the T metabolism is disturbed in UC and Crohn s disease and that excessive losses of T iodine can be a cause of iodine depletion in some patients with longstanding and severe disease.

An earlier study showed evidence suggestive of an increased prevalence of iodine deficiency in patients with ulcerative colitis (UC) or Crohn s disease (10). A hypothesis was put forward that the iodine depletion might be due to increased losses of thyroxine iodine The present study was undertaken to find out if patients with UC or Crohn s disease had alterations of thyroxine (T_4) metabolism in keeping with this hypothesis.

MATERIAL AND METHODS

The experimental group consisted of five patients with UC and six with Crohn s disease The mean age was 31.5 years (range 18–55). As shown in Table I four of the UC patients had extensive or universal disease and one had distal colitis. Of the patients with Crohn s disease three had extensive colonic disease, two widespread small bowel disease and one had ileocaecal inflammation. The diagnosis was based on the clinical history the radiologic findings, the findings at sigmoidoscopy with rectal biopsy and, in six patients, on the findings at subsequent operation together with histologic examination of operation specimens.

Six of the 11 patients had lost considerable weight (3.3–12.7 mean 11 4 kg) and were judged clinically to be severely ill, while the remaining five were mildly or moderately ill. At the time of the study seven f t i were treated with oral or parenteral corticosteroid d them only for the latter half of the investigation None had any clinical symptoms of function l disease and none was receiving any drugs k w influence the thyroxin metabolism apart fr corticosteroids. One patient ran moderat fever time of the study while the others were on-f brile Th patient group was compared with eight health control none of whom was on contraceptives or any othe medic tion, The mean age was 35 9 years (range 27–

The thyroid was first blocked by giving 40 mg potassium iodide orally in two divided doses over the course 4 hours and the blockade was maintained by 60 mg potassium iodide given orally twice daily throughout the tudy period One day after commencing the thyroid blockade blood draw for estimation of T in plasma. This was immediately followed by an i. injection of 10 μCi ^{125}I-T in normal saline

T labelled with ^{125}I in 3 5' position, with specific activity of 20–50 mCi/mg T and a guaranteed content of ^{125}I as free iodide of less than 3 % wa obtained from the Radiochemical Centre Amersham. On delivery it was diluted with 50 % aqueous propylene-glycol in order to reduce the further liberation of free ^{125}I and sterilized by Millipore filtration. Each batch of ^{125}I-T was used within a fortnight. Dr H. Johnston sterilized the ^{125}I-T by ultrafiltration and Dr B. Kågedal Linköping, Sweden, measured the T in serum

The study was performed using the whole-body counter described by Warner and Oliver (27). The value recorded 24 hours after the administration of ^{125}I-T was considered as the 100 % value, thereby allowing time for distribution of the ^{125}I T in the body and also for excretion of ^{125}I-labelled impurities (chiefly free iodide) in the injected sample Each subject was measured on the whole-body counter once daily for 6–10 days and the values were plotted semilogarithmically The biological half-life ($T\frac{1}{2}$) of ^{125}I-T was calculated from the regression line. The fractional daily turnover of ^{125}I-T was calculated from the relation 0.693/$T\frac{1}{2}$ (25). A thyroid count was performed 24 hours after the injection of ^{125}I T and compared with a similar count of the thigh as control tissue. The distribution volume was calculated from the weight, using equation 5 as computed

Table II *Results of the study and statistical calculations*

ETTI = extrathyroidal T_4 iodine. T_4-I = T_4 iodine

	B wt. (kg)	T_4-I/ (2.9–6.3 µg/ 100 ml)	Distribution volume (l)	$T_{1/2}$ (d)	Fractional daily turnover (%)	ETTI pool (µg)	ETTI pool/ 70kg (µg)	T_4-I disposal/d. (µg)	T_4-I disposal/d./70 kg (µg)
I. Mildly or moderately ill patients (n=5)									
Mean	61.7	3.7	10.7	6.14	11.4	389.7	447.3	44.9	51.0
S.D	8.8	0.7	1.2	0.80	1.5	62.9	90.0	10.4	10
S.E.M	3.9	0.3	0.5	0.36	0.7	28.1	40.2	4.6	4.6
II. Severely ill patients (n=6)									
Mean	51.2	3.8	8.8	4.20	17.0	316.8	478.0	51.9	79.0
S.D	21.7	1.8	3.0	0.78	3.4	125.8	253.9	18.8	40.4
S.E.M.	8.8	0.7	1.2	0.32	1.4	51.4	103.6	7.7	16.5
III. All patient (n=11)									
Mean	56.0	3.6	9.7	5.09	14.5	350.0	464.1	48.7	66.3
S.D	17.2	1.3	2.4	1.26	3.9	104.6	189.0	15.3	32.7
S.E.M	5.2	0.4	0.7	0.38	1.2	31.5	57.0	4.6	9.9
IV Controls (n=8)									
Mean	68.0	4.1	11.4	6.88	10.2	464.2	478.2	46.9	48.2
S.D	6.7	1.0	0.7	0.69	1.0	135.8	113.1	13.1	10.7
S.E.M.	2.6	0.4	0.3	0.[illegible]4	0.4	48.0	40.0	4.6	3.8
Significance of the differences									
I–II									
t	1.017	0.121	1.281	4.074	3.379	1.17[illegible]	0.[illegible]3	0.735	1.497
p	N.S	N.S.	N.S	<0.005	<0.01	N.S	N.S.	N.S.	N.S
III–IV									
t	1.772	0.886	1.839	3.627	3.039	1.958	0.178	0.279	1.496
p	N.S.	N.S.	N.S	<0.005	<0.01	N.S.	N.S.	N.S	N.S
II–IV									
t	1.910	0.367	2.191	6.812	5.421	1.920	0.002	0.543	1.909
p	N.S	N.S	<0.05	<0.001	<0.001	N.S	N.S.	N.S	N.S

DISCUSSION

The sensitivity of the whole-body counter used in this study is not dependent on the distribution of the isotope in the body (27). Hence, there is no need to measure the patient in different positions as was done by Oddie et al. (15). It is known that commercial ^{131}I-T_4 contains radioactive impurities, mainly as iodide (26). A certain time is also required after injection for distribution and equilibration of the labelled compound. By using the value 4 hours after injection of ^{131}I-T_4 as the 100% value the influence of radioactive impurities is largely eliminated and the time required for distribution and establishing a state of equilibrium is available. The $T_{1/2}$ values and the daily fractional turnover in the controls are in good agreement with earlier published results as found by the blood disappearance curve (4, 6, 8, 14, 15) and also by whole-bydy counting (15).

Normally about 9–16% of the injected ^{131}I-T_4 is excreted in the faeces within 3–1 days (1, 4, 8, 13, 15). Hence the difference in the frequency of bowel motions between the controls and the patients may influence the results but it is probably of minor importance when the subjects are studied over a period of 6–10 days. This view is supported by the fact that seven of the patients (five of whom had considerably shortened $T_{1/2}$) had only one or two bowel motions daily, so it seems unlikely that diarrhoea as such is the reason for the differences between the patients and the controls.

No appreciable differences were found in the present study between the patients who were treated with corticosteroid and those who were not. Neither did corticosteroids influence the prevalence of iodine depletion in an earlier study of patients with UC or Crohn's disease (10). T_4 metabolism is said to

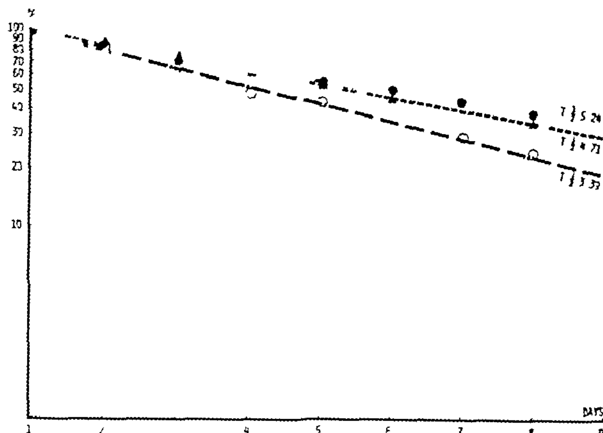


Fig 1 ¹²⁵I thyroxine disappearance curves in three of the patients.

be influenced by corticosteroids, although the re... are rather inconsistent Blomstedt and Ein... 3) found a significant increase in urinary ¹²⁵I ...y after administration of fairly large doses of ...ucosteroids but they also noted a decreased faecal output, even if not of significant magnitude Ingbar and Freinkel (9) found that corticosteroids induced an increased fractional turnover of T and a reduction of its distribution space with an unchanged amount of T iodine disposed of per day Another study on the other hand showed a reduced fractional turnover rate of ¹²⁵I T and a fairly unchanged distribution volume (11). The daily T iodine disposal was reduced in most of the subjects studied. It therefore seems most unlikely that the short T½ and increased daily fractional turnover found in the patients in the present study were due to treatment with corticosteroids

It is well known that many major diseases change the T½ of T (2, 7 12, 20 25) but exactly how the T turnover is regulated is not known. Both UC and Crohn s disease may cause heavy loss of protein through the inflamed gut (28). As T which is protein-bound in plasma, moves across the capillary walls in the same way as albumin (17 4) it is possible that increased amounts of T could be exuded into the gut lumen and there either be excreted in the faeces or be broken down with reabsorption of the iodide which is then partially excreted into the urine It may be relevant that in the nephrotic syndrome considerable amounts of both T and T binding globulin are found in the urine (21 22). A thorough study of the nephrotic syndrome showed that patients with this condition had a small extrathyroidal T iodine pool and a high daily fractional turnover of T (20) The daily disposal of T iodine was about the same as in normal controls but as the amount disposed of in the urine was so large the proportion metabolized by the body was less than normal It was concluded that the increased daily fractional turnover was directly due to the increased losses of T in the urine and faeces In the present study the daily disposal of T iodine was the same in the patients and the controls If a protein-losing enteropathy and blood loss contribute to the losses perhaps a considerable proportion of the T iodine

disposed of per day could be lost in this way It is also possible that a state of iodine depletion could be induced in some patients if the losses were large and the disease of long duration, and even more so if the iodine stores were small at the beginning of the illness. A raised radioiodide uptake which is found in states of iodine depletion was found in about one third of cases of nephrotic syndrome (21) and in 42 % of patients with UC or Crohn's disease (10)

When the daily T iodine disposal in the present study was corrected for weight, three of the 11 patients disposed of more than the mean +2 S.D of the control group. If such a process continues for long a state of iodine depletion can be expected This may explain the finding that 20 % of a group of patients with chronic inflammatory bowel disease had a daily urinary iodine excretion of less than 40 μg (10) In that study it was suggested that the increased prevalence of iodine depletion in UC and Crohn's disease could be due to excessive losses of T and the present study gives support to that concept.

Another possible explanation of the short $T_{\frac{1}{2}}$ of T in UC and Crohn's disease could be an increased demand for *triiodothyronine* (T_3) *It is well known* that T is partly the source of the body T (5 19). Of the daily T production 33 % has been calculated to be converted into T and *this process accounts for* 41 % of the daily T production (18). However preliminary results of a study in progress by the present authors on T metabolism in UC and Crohn's disease have shown the same T turnover rate in patients and controls and at least in some patients a low concentration of T in serum. Therefore the increased T *turnover in these patients does not* seem to be due to an increased conversion of T into T_3.

The present study has shown that T has an increased daily fractional turnover in severely ill patients with UC or Crohn's disease but further work is required to determine the metabolic pathways and routes of excretion.

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Table I *Results and statistical significance of the differences (S.E.M within parentheses)*

	TBG (15–25 μg/100 ml)	TBPA (140–220 μg/100 ml)	Albumin (42 51 g/l)	T_4 (4.9–6.3 μg/100 ml)	T_3-test (80–120%)	Free T_4 index (3 1–6.2 μg/100 ml)
A All patients (n=20)	25.0 (1.6)	84.3 (8 8)	36.3 (1 9)	4 4 (0.3)	106 0 (5 4)	4 4 (0.3)
B Corticosteroid-treated patients (n=10)	21 9 (1.5)	77 7 (14.0)	32.3 (7.3)	3.8 (0.5)	118 0 (7.6)	4 4 (0 5)
C Patients not treated with corticosteroids (n=10)	28 1 (2.5)	90 9 (11.2)	40.3 (2.6)	4 9 (0.3)	94.0 (5.8)	4 4 (0.[illegible])
D Controls (n=20)	19.3 (0 9)	142.8 (7 9)	46.4 (1.4)	4.[illegible] (0.2)	93 8 (3 7)	3 9 (0.2)
Statistical significance of the differences						
A–D	t=3 15 p<0.005	t=4 92 p<0.001	t=4 26 p<0 001	t=0.52 N.S	t=1.85 N.S	t=1 76 N.S.
B–D	t=1.54 N.S.	t=4 05 p<0.001	t=3.26 p<0 001	t=0 73 N.S	t=3.23 p<0.01	t=1.01 N.S
C–D	t=3.31 p<0 005	t=3 79 p<0 001	t=2.07 p<0.05	t=1 7[illegible] N.S	t=0 03 N.S	t=2.12 p<0.05
B–C	t=2 14 p<0.05	t=0 73 N.S	t=2.32 p<0 05	t=1.55 N.S	t=2.50 p<0 05	t=0 08 N.S

Both the corticosteroid-treated patients and those who did not receive such treatment had significantly lower TBPA and albumin values in serum than the [illegible]ols. On the other hand patients receiving [illegible] treatment had significantly lower [illegible] albumin values than patients not receiving such treatment TBPA values did not differ significantly in this respect The correlation between TPBA and albumin was significant when estimated for patients and controls as one group (r=0 620 p<0 001) but insignificant in the patient group alone (r=0 429 N.S) indicating that the two proteins do not behave identically

The T_3-test values were higher in the patients than in the controls but the difference was not significant. Treatment with corticosteroids resulted in significantly higher T_3-test values compared to the controls and also to the patients not receiving such treatment.

The free T_4 index was similar in the patient and the control groups. Patients who did not receive corticosteroid treatment had significantly higher free T_4 index than the controls. However in the total patient group only one patient with a free T_4 index of 8 7 had a value above the upper normal limit.

The TSH level was normal in all patients (mean 4 9 range <3–10 μU/ml) and in all controls (mean 6.2 range <3–10 μU/ml).

DISCUSSION

Thyroxine in serum is normally bound by TBG (75%) TBPA (15%) and albumin (10%) (28) Hypoalbuminemia is a well known consequence of UC and Crohn s disease due to increased faecal losses of albumin (27) This was probably the cause of the low serum albumin in the present patients

TBPA is usually decreased in systemic diseases (1 11 15 17 22) The decreased TBPA concentration has been attributed to decreased synthesis (15 25) but also in some cases to increased degradation (18) As protein-bound T_4 moves across the capillary walls in the same way as albumin (16, 24) it is possible that increased amounts of TBPA could be exuded into the gut lumen and lost in the faeces If so both decreased synthesis and increased degradation could be the cause of the reduced concentration of TBPA in UC and Crohn s disease

The reason for the increase in TBG is not known as it is usually reported to be normal or reduced in systemic diseases with the exception of liver dis-

ease and acute intermittent porphyria, in which raised TBG values have been noted (6 12) Liver disease is not infrequent in patients with UC or Crohn s disease. However usually the histological liver changes are mild and the liver is grossly diseased only in a small proportion of the patients (20 21). The present patients were not thoroughly investigated for the occurrence of liver disease but none of them had any clinical evidence of severe liver disease and bilirubin, transaminases and alkaline phosphatase in serum were normal

Raised TBG and TBPA values have also been reported in a patient with analbuminemia with reversion to normal when the serum albumin concentration was restored (5) In the present study three patients with high TBG values (37–40 μg/100 ml plasma) had normal albumin values indicating that the increase in TBG is not a compensatory phenomenon in UC and Crohn s disease.

Increased TBG concentrations have been reported in such geographical areas where iodine deficiency is common (26). Evidence of iodine deficiency is fairly common in UC and Crohn s disease (8), but our patients were not investigated for the occurrence of iodine deficiency

No definite explanation of the increased concentration of TBG in UC and Crohn s disease can be given at present, but subclinical liver disease interfering with estrogen metabolism cannot be ruled out. This may be supported by the fact that the highest TBG values were found in female patients On the other hand iodine deficiency which is more common in women, cannot be ruled out either

The increase in TBG appears to compensate for the decrease in binding sites for T on TBPA and albumin as the T concentration in serum for both the patients and the controls was almost identical T_4, which otherwise would be bound to TBPA or albumin, is now probably bound to TBG This would imply that a relatively greater proportion of the T binding sites on TBG are occupied than normally The values from the T_3-test, which reflects the number of free binding sites on TBG were also slightly although not significantly higher in the patients than in the controls. This would also help to preserve a normal concentration of free T in the serum, and the normal TSH values indicate that the concentration of free T is at least not subnormal On the contrary the f ee T concentration in serum may be slightly higher in patients with UC or Crohn disease who are not treated with corticosteroid than in healthy controls if the free T index truly reflects the free T concentration in serum

Corticosteroids are known to increase TBPA and decrease TBG (10 19) This is partly confirmed in the present study as corticosteroid-treated patients had significantly lower TBG values than patients who did not receive such treatment. On the other hand the controls and the corticosteroid-treated patients had the same TBG levels, indicating that corticosteroid treatment in UC and Crohn s disease induces a reduction of an otherwise increased TBG synthesis so that the TBG level becomes normal This phenomenon together with the decreased concentrations of TBPA and serum albumin is probably the explanation of the slightly decreased T values in corticosteroid-treated patients compared to those not receiving such treatment. That corticosteroids reduce the T and increase the T_3-test values is well known (2).

Patients treated with corticosteroids had a significantly lower serum albumin than the patients who did not have such treatment, while the TBPA values were lower but not significantly so As corticosteroids increase the TBPA one would have expected these values to be higher in the corticosteroid-treated group However there is reason to believe that the corticosteroid-treated patients were more severely ill than the patients who did not receive such treatment. As larger amounts of proteins are lost via the faeces in patients with severe inflammatory bowel disease than in patients with moderate or mild severity of the disease this could be the reason why the corticosteroid-treated patients had lower TBPA and albumin in serum than the patients not receiving such treatment. It is also possible that this could contribute to the lower TBG values in corticosteroid-treated patients compared to those who did not have such treatment.

The findings presented here show that in UC and Crohn s disease changes in the concentrations of the thyroid hormone binding proteins can occur which sometimes may cause alterations in the thyroid tests so that hyperthyroidism can be suspected In a patient with diarrhoea weight loss and sometimes anxiety which all are common findings in UC and Crohn disease a raised T and T_3-test value make a misdiagnosis of hyperthyroidism easy As a thyroid radioiodide uptake test often shows uptak values within the hyperthyroid range probably due to iodine deficiency such a misdiagnosis [illegible] even to make (8) This condition has

"pseudo-hyperthyroidism (7) and a further example of this condition is provided by one of the patients in the present series with a T I level of 7 0 μg/100 ml and a T_3-test value of 124% His free T index of 8 7 was also high After treatment of his bowel disease the thyroid tests became normal

There is an increased prevalence of hyperthyroidism in patients with UC (9) Hence in patients with UC or Crohn s disease in whom the thyroid tests give rise to a suspicion of hyperthyroidism, which is not clinically clear-cut, it is necessary to perform a triiodothyronine suppression test in order to confirm or rule out a diagnosis of hyperthyroidism

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INHIBITION OF ADRENAL FUNCTION IN MAN BY HEPARIN OR HEPARINOID Ro 1-8307

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Abstract Heparin and the heparinoid Ro 1-8307 inhibited the secretory rate of aldosterone in physiological or pathological aldosteronism to the level found in normal subjects on liberal sodium intake. In addition, these compounds inhibited corticosterone biosynthesis, although less markedly than that of aldosterone Indications of interference with cortisol production have not been found. During drug treatment angiotensin, in doses of 5–10 ng/kg b.wt /min, did not stimulate aldosterone secretion. ACTH responsiveness of the adrenals—indicated by the fractional increases of both aldosterone and corticosterone secretory rates—remained unchanged. I two studies heparin had no consistent effect on plasma renin activity

Heparin and related polysulphated polysaccharides like Ro 1-8307 (N-formyl-chitosan polysulphuric acid) have been shown to produce natriuresis and potassium retention (300–400 mg of regular heparin being equivalent to 600–800 mg of Ro 1-8307) (24 25 27 31 32) These effects proved to be caused by a decrease of the excretory and secretory rate of aldosterone both in normal subjects on low sodium intake and in patients with secondary (5 13 17 22, 23 31 37) and *primary aldosteronism (1 15 16 19)*

The mechanism by which these substances act on adrenal steroidogenesis in vivo is by no means clear Decreased secretion of 18-hydroxycorticosterone has been observed by Abbott et al (1) to occur simultaneously with decreased aldosterone secretion during treatment of hypertensive subjects with Ro 1-8307 Therefore these authors concluded that the site of action of this heparinoid in adrenal *steroidogenesis is prior to the synthesis of 18*-hydroxycorticosterone. Conn et al (15) observed decreased excretion of an 18-hydroxycorticosterone metabolite 18-hydroxytetrahydro-11-dehydrocorticosterone during administration of Ro 1-8307 to three patients with primary aldosteronism simultaneously the excretory rate of a corticosterone metabolite tetrahydrocorticosterone was reported to remain unchanged. These investigators speculated that the heparinoid might block the conversion of corticosterone to 18-hydroxycorticosterone. As a consequence they reasoned that normal subjects treated with the heparinoid, should have a higher rate of corticosterone production than controls. This would occur under these conditions because of increased activity of the aldosterone biosynthetic pathway due to the sodium *loss caused by the treatment with the heparinoid*

In this study observations are reported of the effect of heparin and Ro 1-8307 on the secretory rates of aldosterone and corticosterone the plasma renin activity (PRA) and the adrenal responsiveness to ACTH and angiotensin

MATERIAL AND METHODS

Clinical

Fourteen normal subjects or patients without discernible disturbances of the renin angiotensin aldosterone system, five normal subjects or patients with secondary aldosteronism due to natriuretic treatment and two patients with deep femoral vein thrombosis treated with the conventional anticoagulant, acenocoumarol (Sintrom, Geigy Basle Switzerland) and diuretics participated in this study

In all the studies the patients maintained a constant diet containing about 15 mmol sodium. Urine collection was checked daily for completeness by determination of creatinine content, and the excretion of sodium and potassium was measured. After equilibration aldosterone and corticosterone secretory rates and the influence of heparin or Ro 1-8307 (prepared and provided by Hoffmann-La Roche Laboratories, Basle Switzerland) on these secretory rates were determined *Heparin (Thromboliquin* Organon Oss, The Netherlands) (200–400 mg daily) was given i   and the heparinoid Ro 1-8307 (400–800 mg daily) m. or i. ., both in divided doses. I   number of

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studies the effects of angiotensin or ACTH were determined. These polypeptides were given by continuous i. infusion over a 4-hour period angiotensin (Hypertensin Ciba, Basle Switzerland) in doses of 5–10 ng/min/kg b wt. ACTH (ACTH-Organon, Oss, The Netherlands) in a total dose of 120 U

In two studies aldosterone secretory rates and PRA were measured repeatedly on alternating days before, during and after heparin treatment. All aldosterone and corticosterone secretory rates were measured in patients during 24 hours of recumbency PRAs were measured in blood taken at noon after 3 hours of quiet ambulation or sitting in chair

Laboratory procedures

Aldosterone secretory rates were determined by an earlier described double isotope dilution derivative technique (6, 7). In 40 normal subjects on a dietary sodium intake of about 115 mmol a mean value of 135±49 (1 S D) μg/24 h was found.

Corticosterone secretory rates were also measured by double isotope dilution derivative method (11). This method differs from Peterson and Pierce s (29) by the introduction of thin layer chromatography Four μCi of 1,2-^{3}H-corticosterone (New England Nuclear Corporation) is injected i. After treating an aliquot of the 24-hour urine with β-glucuronidase the dichloromethane extract is chromatographed on a silica gel layer twice in the system chloroform–ethanol, 99 1 (/v) and once in the system chloroform–methanol–water 94 6 0.5 (/v). The area which contains the radio-labeled corticosterone metabolites, tetrahydro- and allotetrahydrocorticosterone is eluted and chromatographed on second silica gel layer in the system chloroform–acetone–water 60 4 0.5 (/v) Separation of the two metabolites is achieved by paper in the system cyclohexane–benzene–water 100 100 100 50 (v/). One metabolite acetylated with ^{14}C-acetic anhydride 0.2 mCi/mmol. diacetate formed is purified by thin layer chromatography in the system chloroform–acetone 98 2 (v/v), subsequently by paper chromatography in the system cyclohexane–benzene–methanol–water 100 25 100 10 (/v) and finally by thin layer chromatography in the system chloroform–acetone, 95 5 (/v). Purity of the acetate i assessed by comparing the ^{3}H/^{14}C ratios after each of the last two chromatographic steps. In a number of determinations the method was checked by comparing the ^{3}H/^{14}C ratios of the diacetates of both metabolites and by hydrolysis of the diacetates of one metabolite into its monoacetate. By the last procedure the ^{3}H/^{14}C ratio was increased by hydrolysis was 2.02±0.09 (1 S.D). In 16 normal subjects a mean secretion rate of 3.8±1 0 μg/24 h was found.

PRA wa measured by the method of Boucher et al. (9) in which an additional purification step was introduced. After neutralization of the angiotensin-containing eluate of the dowex column, the aqueous solution is saturated with sodium chloride and subsequently extracted with butanol. Angiotensin is back-extracted from the organic layer into dilute hydrochloric acid and the acid solution evaporated under reduced pressure. The residue is taken up in albumin-containing buffer just before the bioassay is performed. The relative S.D. at level of 480 ng angiotensin/3 h/100 ml was 8.5% (16 determinations) and 8.0% at a level of 1 100 ng/3 h/100 ml (11 determinations). Normal values for persons on diet containing about 115 mmol sodium were 189±115 (1 S.D.) ng/3 h/100 ml in the supine and 589±461 in the upright position (15 determinations) Urinary 17-hydroxycorticosteroids were measured by the method of Appleby et al. (4) urinary 11-hydroxy corticosteroids by the method of Mattingly et al. (28) urinary 17-ketosteroids by the method of Callow et al (10). Urinary sodium, potassium and creatinine were determined by routine laboratory methods (20)

Statistical methods

The differences in data between groups were tested by Student s two-sample test.

RESULTS

The effect of heparin and Ro 1-8307 on aldosterone and corticosterone secretory rates

Aldosterone secretory rates decreased during administration of heparin or Ro 1-8307 both in normal subjects and in patients with aldosteronism. On the first day of each course the secretory rate did not change significantly in 6 subjects (nos. 1–6) without demonstrable disturbances of the renin-angiotensin-aldosterone system (Table I) the mean secretory rate before treatment being 415±197 (1 S D) against 468±183 (1 S.D) on the first day (p>0.1) On the second day lower values were measured than before treatment. On the 4th or 5th day of heparin or Ro 1-8307 the secretory rates in 6 normal subjects (nos. 7–12) averaged 35% (range 16–62) of the pretreatment control values (Table I)

In patients with cardiac failure and aldosteronism due to diuretics (nos. 13–15) a steady decrease in aldosterone secretory rates was found during prolonged administration of heparin or Ro 1-8307 starting on the second day and falling to about 20% of control values (Table I)

Remarkably 4 or 5 days heparin or Ro 1-8307 treatment of normal subjects on sodium-restricted diet decreased aldosterone secretion to levels found in normal subjects on liberal sodium intake the mean 119±35 μg/24 h during administration of these drugs is though lower not significantly different from the mean value found in this laboratory in normal subjects on a dietary sodium intake of about 115 mmol (p>0 1). The data of this study do not exclude the possibility that heparin or Ro 1-8307 given during longer periods might decrease the aldosterone secretory rates even more in normal subjects The observations in patients 13 and 14

Table I *Effect of heparin or Ro 1-8307 on aldosterone and corticoste one secretory rates in normal subjects (nos 1–12) and pati nts with secondary aldosteronism (os 13–15) on low sodium intake (about 15 mmol daily)*

Subj. no.	No of days of treatment[a]	Aldosterone secretory rat (µg/24 h) Before treatment	During treatment[b]	After treatment[b]	Corticosterone secretory rate (mg/24 h) Before treatment	During treatment[b]	After treatment[b]
1	1 H	342	418 (1)	291 (2)	3.0	3 0 (1)	_4 (2)
2	1 H	665	550 (1)	368 (?)	6.9	7 (1)	6 3 (2)
3	1 H	638	791 (1)	757 (2)	7.6	7 1 (1)	5 1 (2)
4	1 H	370	43? (1)	49 (3)	_9	3 1 (1)	3 4 (3)
5[c]	1 R	_37	281 (1)				
	2 R	_34	133 (2)				
6[c]	1 R	200	339 (1)				
	2 R	180	173 (2)				
7	4 H	233	170 (4)	398 (4)			
		312					
8	5 H	345	128 (5)	546 (4)			
9	6 H	295	73 (4)	275 (4)			
		244					
10	6 H	331	137 (4)	280 (4)	5_	3.3 (4)	6 4 (4)
		_38			5 6		
11	7 H	545	85 (5)	333 (4)	3 8	0 (5)	3 7 (4)
			70 (7)		3 0	1 4 (7)	
12	6 R	799	121 (4)	764 (4)	5.2	2.8 (4)	4 7 (4)
		690			6.5		
13[d]	8 H	1 265	587 (2)				
		1 253	425 (4)				
			197 (8)				
14[d]	9 R	525	423 (2)	70 (4)			
		630	220 (4)	257 (8)			
			75 (9)				
15[d]	11 H	919	200 (10)	738 (6)			

H=heparin, R=Ro 1-8307 Parentheses indicate no of days Aldosterone secretory rates were determined over 12-h periods. Secondary aldosteronism due to diuretic treatment of cardiac failure

with heart failure and secondary hyperaldosteronism due to diuretics illustrate that this may occur

Corticosterone ecretory rat were found to be decreased during heparin or Ro 1-8307 treatment in 3 subjects (nos. 10–12) (Table I). Values of about one half of pretreatment control values were found on the 4th–7th day of drug administration. On the first day of treatment the secretory rate did not change appreciably (nos. 1–4) (Table I).

In these studies aldosterone and corticosterone secretory rates were measured simultaneously both before and during courses of heparin or Ro 1-8307 injections. Table II summarized the percentages of inhibition of aldosterone and corticosterone secretory rates in the studies in which the drugs were given during at least 3 days. The mean percentage of inhibition of the aldosterone secretory rate was significantly higher ($p>0\ 05$) than that of corticosterone

Recovery after drug thdraw l On the 4th day after withdrawal of heparin or the heparinoid the aldosterone secretory rates n 6 normal subjects (nos 7–12) were similar to the control values the mean after w thdrawal (433±191 µg/24 h) being not significantly different ($p>0\ 1$) from the corresponding pretreatment level (410±195 µg/24 h) The corticosterone secretory rates also returned to the control values the mean after treatment being equal to the mean before (4 9 mg/24 h) (Table I)

The effects of a giotensin and ACTH on the ecretory rates of aldosterone and corticosterone before and during administration of heparin or Ro 1-8307

Angiotensin (5–10 ng/kg b wt./min i over 24 h) d'd not elicit rise in *aldosterone* secretory rate on the 6th or 7th day of heparin administration (subjects 8 and 9). Before heparin the same amount of angiotensin increased the aldosterone secretory rate significantly ($p<0\ 005$). The stimulatory effect of angiotensin was also absent on the first day of heparin or Ro 1-8307 injections in two subjects (nos. 16 and 17) with aldosteronism due to administration of

Table II *Percentage inhibition of simultaneously measured aldosterone and corticosterone secretory rates during treatment with heparin or Ro 1-8307*

Subj. no.	Percentage inhibition: Aldosterone secretory rate	Corticosterone secretory rate	Treatment[a]
10	52	39	Heparin (4)
1	84	40	Ro 1-8307 (4)
11	84	47	Heparin (5)
11	87	63	Heparin (7)
Mean	76.8	47.2	
		$p<0.05$	

[a] No of days within parentheses.

diuretics (Table III) Fig. 1 illustrates the data of one of the subjects in detail The natriuretic effect of heparin is evident. The BP response elicited by angiotensin was about equal before and during drug treatment.

ACTH responsiveness of the adrenals was tested in several studies (Tables *IV* and V) A total amount of 120 U was administered in a continuous i v infusion over 24 hours. In two normal subjects (nos. 5 and 6) the *effect* of ACTH on the secretory rate of *aldosterone* was tested twice, once on the 1st and on the 2nd day of a course of Ro 1-8307 injec (Table IV) Attention is drawn to the fact that observations were made in different periods the study in order to avoid interference with the well known diminishing effect of successive doses of ACTH on aldosterone secretion (8 1 22, 34).

The data of Table IV illustrate that the stimulatory effect of ACTH on the first day of treatment with heparin or Ro 1-8307 (subjects 5 and 6) was quantitatively similar to the effect of the same dose of ACTH before the administration of these drugs (subjects 10 and 12) Observations later in the study periods show that the absolute amounts of aldosterone produced in response to ACTH fall clearly after more days of heparin or Ro 1-8307 However with regard to the appropriate control values the fractional increases before and during administration of these *drugs* were almost equal.

Comparing the effects of angiotensin and ACTH (Tables III and IV Figs. 1 and 2) a remarkable difference between the responses to these stimuli during heparin or Ro 1-8307 was found. At the chosen dose both stimuli increased the aldosterone sec retory *rate* before administration of the sulphated polysaccharides by one and a half to threefold. Neither on the *first* nor on following days of heparin or heparinoid however was a response to angiotensin *discernible* whereas during ACTH a frac tional increase of the aldosterone secretory rate was found similar to that before treatment with the polysaccharides

The data of Table V on the effect of *ACTH* on the *corticosterone* secretory rate before and during heparin or Ro 1-8307 present the same pattern as Table IV shows for aldosterone Although the amount of corticosterone produced by stimulation with ACTH was clearly less during than before drug administration the fractional increase was

Table III *Effect of angiotensin (5–10 ng/kg/min during 24 h i v) on aldosterone secretory rates in normal subjects and patients with secondary aldosteronism on low sodium intake (about 15 mmol daily) before and during treatment with heparin or Ro 1-8307*

Subj. no.	Aldosterone secretory rate (µg/24 h)[b]: Control before treatment	Angiotensin before treatment	Angiotensin during 1st day of treatment	Control during 4th–5th day of treatment	Angiotensin during 6th–7th day of treatment	Control after treatment	Treatment
Normal subjects							
8	345 (4)	658 (6)		128 (11)	117 (13)	546 (17)	Heparin
9	295 (6)	587 (8)		73 (15)	62 (17)	275 (21)	Heparin
18	233 (5)	522 (7)					
19	485 (5)	547 (7)					
Secondary aldosteronism[a]							
16	347 (5)	1 604 (7)	764 (10)				Heparin
17	1 100 (4)	1 548 (6)	1 154 (11)				Ro 1-8307

[a] Due to sodium depletion by natriuretic treatment. [b] Parentheses indicate no of days of study period

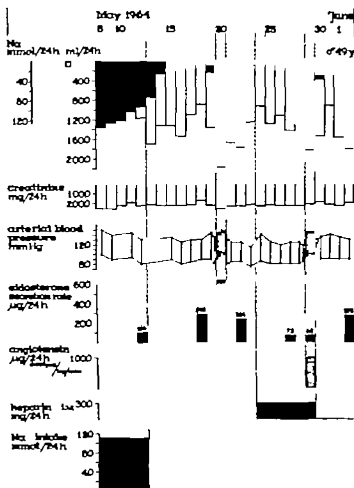


Fig. 1. Effect of angiotensin on the aldosterone secretory rate in normal subject (no. 9) on a constant 15 mmol sodium diet before and during heparin treatment. During treatment the aldosterone secretory rate decreased and sodium loss occurred. Before treatment angiotensin increased the secretory rate of aldosterone. During treatment angiotensin did not stimulate aldosterone secretion. *Note that the BP response to angiotensin was about equal before and during heparin treatment.*

Table IV *Effect of ACTH (120 U/24 h i) on aldosterone secretory ate in normal subjects on low sodium intake befor and during treatment ith heparin or Ro 1-8307*

In subject 5 and 6 the secretory rates were determined over 12-h periods. The effect of ACTH in these subjects was studied in two separate courses of Ro 1-8307 treatment. In subject 5 the first course started on day 13 and the second on day 17. In subject 6 the start of the separate courses was on days 11 and 15

Subj. no.	Aldosterone secretory rate (µg/24 h)ᵃ Control before treatment	ACTH before treatment	ACTH during 1st day of treatment	ACTH during 2nd day of treatment	Control during 4th day of treatment	ACTH during 6th day of treatment	Control after treatment	Treatment
5	237 (7)							Ro 1-8307
	234 (12)		710 (13)	430 (18)				
6	200 (5)							Ro 1-8307
	180 (10)		1 097 (11)	337 (16)				
10	331 (5)	1 104 (7)						Heparin
	238 (10)				137 (15)	262 (18)	280 (21)	
12	799 (5)	1 768 (7)						Ro 1-8307
	690 (17)				121 (16)	.31 (18)	764 (22)	

Parentheses indicate no. of days of study period

Table V *Effect of ACTH (120 U/24 h i v) on corticosterone secretory rate in two normal subjects on low sodium intake before and during treatment with heparin or Ro 1-8307*

	Corticosterone secretory rate (mg/24 h)					
Subj. no.	Control before treatment	ACTH before treatment	Control during 4th day of treatment	ACTH during 6th day of treatment	Control after treatment	Treatment
10	5.2	51.0	3.3	39.0	6.4	Heparin
12	5.2	104.0	2.8	45.0	4.7	Ro 1-8307

equal in both experimental situations Fig. 2 shows one of these observations in detail (subject 12)

The effects of heparin or Ro 1-8307 on the urinary excretion of other corticosteroids than aldosterone and the effect of ACTH upon these excretions before and during administration of these drugs

In two of the subjects studied the excretory rates of both 17 and 11-hydroxycorticosteroids were measured on successive days before and during courses of heparin or Ro 1-8307 Table VI shows that the excretion of these steroids was not changed by treatment with either agent. Table VII illustrates that ACTH increased the excretory rates of both steroid groups during administration of the polysulphated polysaccharides to levels comparable with those before treatment In addition the effect of ACTH on the excretory rate of 17-ketosteroids dur

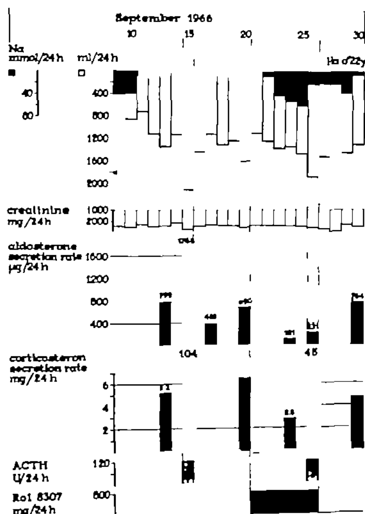


Fig 2 Effect of ACTH on aldosterone and corticosterone secretory rates in subject 12 on constant 15 mmol sodium diet before and during treatment with Ro 1-8307 During treatment both secretory rates decreased and natriuresis occurred. The fractional increase in both aldosterone and corticosterone secretory rates by stimulation with ACTH was about equal before and during Ro 1-8307 treatment. Note that during treatment ACTH inhibited drug-induced natriuresis.

Table VI *Effects of heparin or Ro 1-8307 on th excr tory ates of urinary 17 and 11-hydroxy ortico-steroids (mean ± 1 S.D)*

Subj no.	17-hydroxycorticosteroids (mg/24 h) Before treatment	During treatment		11-hydroxycorticosteroids (μg/24 h) Before treatment	During treatment	
10*	9 4±1 7 $N=5$	9 7±2.2 $N=6$	s.	178±31 $N=8$	199±35 $N=5$	n.s.
12°	16.2±3 0 $N=9$	15.8±1 2 $N=5$	s.	44±65 $N=8$	206±58 $N=5$	n.s.

Heparin treatment. Ro 1-8307 treatment. n.s. = not significant

ing heparin or Ro 1-8307 was almost identical to that before administration of these drugs although the control values during injections of the sulphated polysaccharides were lower than in the pretreatment period.

The effect of heparin on the activity of the renin-angiotensin sy tem

In two male subjects with femoral vein thrombosis (nos. 20 and 21) the effect of heparin administration on PRA was studied before, during and in one individual, after administration of the drug (Table VIII). Aldosterone secretory rates were obtained on alternating days Heparin was injected during 5 days in the first and 7 days in the second study the dose ranging from 220 to 350 mg/day In the first study sodium loss during heparin amounted to about 330 mmol In this period PRA did rise very slightly After drug withdrawal however the PRA values appeared to be about twice the pretreatment level. For unknown reasons the pretreatment aldosterone secretory rates in this subject were higher than those normally found during sodium restriction in this laboratory In the second study sodium loss during the 7 days of heparin injections amounted to 270 mmol In this observation PRA showed an equivocal decrease. In both studies the aldosterone secretory rate decreased distinctly to 22 and 28 % of the pretreatment control values respectively

DISCUSSION

Heparin and the chemically related polysulphated polysaccharide Ro 1-8307 inhibited, after treat ment during 4–5 days the secretory rate of aldosterone in normal subjects on low sodium intake and in patients with aldosteronism to levels that are found in subjects on liberal sodium diets confirming our earlier observations (22) and those of Bailey and Ford (5) This adrenal inhibitory action became apparent on the second day of treatment with these drugs. The natriuretic action of these drugs is also

Table VII *Effect of ACTH on the xcretory rates of 17 nd 11-hydroxy ortico teroids nd 17 keto-steroids before and during treatment ith heparin or Ro 1-8307*

The control values represent the excretory rates on two successive days, ACTH values are those of the day of ACTH administration and the day thereafter

Subj no	17-hydroxycorticosteroids (mg/24 h) Before treatm Control	Before treatm ACTH	During treatm Control	During treatm ACTH	11-hydroxycorticosteroids (μg/24 h) Before treatm Control	Before treatm ACTH	During treatm Control	During treatm ACTH	17-ketosteroids (mg/24 h) Before treatm Control	Before treatm ACTH	During treatm Control	During treatm ACTH
10*	10	42.9	13 3	6_7 (7)	240	4 330	210	6 010 (7)	18.1	31.6	9.5	27 1 (7)
	9	50.4	7 8	80.1	160	3 120	160	3 870	13 1	35 7	4 9	36 8
12°	19 0	87 9	16 7	81 2 (6)	220	5 820	200	2 310 (6)	13 5	29 8	7 8	22 7 (6)
	11 4	69 0	14 9	85 7	170	260	150	1 440	10 8	4 3	9 8	28.5

Heparin treatment. Ro 1-8307 treatment Parentheses indicate no. of days of drug treatment period

Table VIII *Effect of heparin on PRA and aldosterone secretory rate*

Subj. no	PRA (ng/100 ml/3 h) Before treatment	During treatment	After treatment	Aldosterone secretory rate (µg/24 h) Before treatment	During treatment	After treatment
20	675 (3)	700 (3)	1 125 (2)	1 267 (4)		318 (1)
	625 (5)	825 (5)	1 200 (3)	983 (2)	214 (4)	1 350 (4)
			1 200 (5)			
1	600 (3)	350 (2)		288 (4)		551 (11)
	400 (5)	250 (4)			84 (3)	
		400 (7)			75 (6)	

Parentheses indicate no. of days before, during and after treatment, respectively

delayed until the second day of treatment or even longer (13 22, 4 27 31). This inhibition of adrenal steroidogenesis is completely reversible after drug withdrawal The same pattern of inhibition was shown for corticosterone. However the inhibition of the secretory rate of this corticosteroid appeared to be weaker than that of aldosterone (11 12).

During heparin or heparinoid treatment the responsiveness of the zona glomerulosa to ACTH—indicated by the fractional increase of the aldosterone secretory rate— was still present and comparable with the effect of ACTH in normal subjects on liberal sodium intake (22, 34) Corticosterone secretion during heparin or Ro 1-8307 treatment was stimulated by ACTH in a similar way the fractional increase being about equal before and during administration of the sulphated polysaccharides However exogenous angiotensin—unlike ACTH—did not stimulate aldosterone secretion discernibly during courses of heparin or heparinoid at least not at the chosen dose levels. One might argue that, if no angiotensin had been given, the aldosterone secretory rate would have been lowered further in response to continued treatment with heparin or its congener so that lack of response to angiotensin could be the result of inhibition by a polysulphated polysaccharide and stimulation by angiotensin. Arguing against such an interpretation is the fact that, during treatment with heparin or Ro 1-8307 aldosterone secretory rates were never lower than those found in normal subjects on liberal sodium intake, as is illustrated by the values of subjects 8 and 9 in Table III At this point it should be added that in two observations not reported in this paper the effect of similar amounts of angiotensin was studied in two subjects on liberal salt intake. Angiotensin increased aldosterone secretory rates in these two subjects from 94 and 150 µg/24 h to 440 and 228 µg, respectively Although the number of observations is too small to permit definite conclusions these data suggest that heparin and Ro 1-8307 inhibit selectively the action of angiotensin in the zona glomerulosa, whereas ACTH remains stimulatory with the zona glomerulosa working at a lower gear

From the few observations reported in the literature it appears that during heparin or heparinoid treatment PRA increases (5 30). From these and the two studies reported here in which no consistent effect on PRA during heparin treatment was observed it is concluded that aldosterone inhibition by heparin is clearly not mediated by renin suppression

So far no indication of interference with cortisol production has been found. The excretory rate of 17 and 11-hydroxycorticosteroids did not change during heparin or Ro 1-8307 treatment in accordance with earlier reported data (1 27 37) These findings per se do not rule out partial inhibition of the zona fasciculata function as compensatory release of ACTH might rapidly abolish such an inhibitory effect (18) The results of an earlier study (27) in which urinary 17-OH-corticosteroid excretion, stimulated by ACTH did not change distinctly during addition of heparin but rose significantly when heparin was omitted and ACTH continued do suggest that under special circumstances heparin can influence the function of the zona fasciculata. In fact the decrease in urinary excretion of 17-ketosteroids as reported earlier (26, 36) and reconfirmed by the observations in this study could mean that heparin and Ro 1-8307 interfere with adrenal function in a rather unselective way However our observation that exogenous ACTH increased the excretory rates of 17-hydroxycorticosteroids, 11-hydroxylated compounds and 17-ketosteroids during drug treatment to

the same extent as in the pretreatment period suggests that the main effect of short-term administration of heparin or Ro 1-8307 is confined to the zona glomerulosa. This explanation is in accordance with the histological findings in rats treated with these drugs. Vallent et al. (35) reported a decreased width of the zona glomerulosa of salt-depleted rats after administration of heparin. Abbott et al (2 3) found atrophy of the zona glomerulosa of rats on normal sodium intake without clearcut alterations in the zona fasciculata after treatment with Ro 1-8307 and Chremos et al. (14) obtained identical results in regenerating rat adrenal glands and in intact adrenals of sodium-deprived rats

In this context the observation by Wilson and Goetz (39) may be mentioned These authors reported isolated hypoaldosteronism and at post mortem examination, atrophy of the adrenal zona glomerulosa in a 38-year-old man who was treated for severe arteriosclerosis and hypercholesterolaemia with intramuscular heparin injections during 4 years.

Data of in vitro studies indicate that heparin can interfere with the biosynthesis of aldosterone. Sharma et al. (33) studied the conversion of labeled corticosterone into aldosterone in a mitochondrial preparation of frog adrenals. These investigators found that, in the absence of calcium in the incubation medium, heparin inhibits this conversion in rather low concentrations (10^{-4} mol) Benraad et al. (unpublished observations) studied the conversion of labeled progesterone into corticosteroids by slices of an aldosteronoma and by beef and rat adrenals At a higher concentration (10^{-4} mol) than used by Sharma et al heparin caused a distinct inhibition of the conversion into corticosterone 18-hydroxycorticosterone and aldosterone with concomitant accumulation of deoxycorticosterone. Both these in vitro studies indicate that heparin or Ro 1-8307 interferes with the biosynthesis of aldosterone, the latter providing additional evidence that also corticosterone synthesis is inhibited by these drugs Together these findings might reflect our results in clinical studies, showing inhibition of biosynthesis of both aldosterone and corticosterone

The combination of unresponsiveness of the zona glomerulosa to angiotensin with maintenance of responsiveness of ACTH found during heparin or Ro 1-8307 treatment, is rather unique effect of an adrenal inhibitory drug Angiotensin as well as ACTH are considered to be physiological regulators of mineralocorticoid production (38) Their relative importance for aldosterone secretion in man is still a matter of dispute and their mechanisms of action are still unclear Polysulphated polysaccharides such as heparin or Ro 1-8307 might be useful tools in the unraveling of these problems.

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INSULIN RELEASE IN FASTING MAN INDUCED BY IMPURE BUT NOT BY PURE PREPARATIONS OF CHOLECYSTOKININ

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Abstract Most preparations of cholecystokinin reported to release insulin have been impure When highly purified preparations of extracted cholecystokinin and also the synthetic C-terminal octapeptide of the hormone became available for use in humans, we investigated their insulinotropic activity in comparison with cruder preparation of cholecystokinin in 10 fasting non-diabetic subjects. The doses employed were 75 Ivy dog units, except for the synthetic C-terminal octapeptide of cholecystokinin that was given in a dose of 200 Ivy dog units to compensate for a shorter half-life. Blood samples for determination of insulin and glucose were drawn every minute during 10 min after each injection, thereafter at intervals of 5 min. The mean plasma insulin level increased significantly reaching peak 4–5 min after i. injection of the cruder cholecystokinin preparation, but after the other two preparations the plasma insulin level was not significantly changed. The blood glucose level was not significantly changed by any of the preparations used. It is concluded that the plasma insulin peak seen in man after v injection of the less highly purified preparation was due not to cholecystokinin but to some other agent present in this less pure preparation. The identity of this factor discussed

In 1964 McIntyre et al. (16) demonstrated that hyperglycemia produced by oral glucose caused a greater release of insulin in man than a comparable degree of hyperglycemia produced by i.v glucose In the same year Dupré (6) found that a crude extract of intestinal mucosa improved i.v glucose tolerance These observations drew the attention to the possibility that during the resorption of glucose the gastrointestinal tract might produce a humoral factor that enhanced the release of insulin from the β-cells. The structure of this insulinotropic hormone is still not known, but an ability to release insulin has been ascribed to several of the known gastrointestinal hormones including cholecystokinin-pancreozymin.

However the reports on the insulinotropic activity of cholecystokinin in man are contradictory Thus Raptis et al (18) and Adlung and Uthgenannt (1) found a plasma insulin peak after i.v administration of cholecystokinin, while Dupré and Beck (8) found no such effect of the hormone The results of these investigations may however be difficult to interpret due to the use of impure preparations of cholecystokinin and recently Creutzfeld (5) stressed the importance of using pure and homogenous preparations when investigating the insulinotropic effect.

We had the opportunity to obtain pure and well defined preparations of cholecystokinin, namely a highly purified extracted preparation, and the synthetic C-terminal octapeptide of the hormone. Therefore we investigated the ability of these preparations of cholecystokinin to release insulin in man as compared to a less highly purified preparation.

MATERIAL AND METHODS

Ten non-diabetic subjects, six men and four women aged 20–45 years, were investigated.

The subjects had fasted overnight. On the following morning cubital vein of each arm was cannulated using indwelling plastic catheters with stopcocks One of the catheters was used for injection of the test substances, the other one for blood sampling The catheters were perfused with saline after each blood sampling.

The three test preparations were given i at intervals of 30 min 3 ml blood were drawn into test tubes containing EDTA to prevent coagulation 15 and 5 min before the first injection After the injection of test substance had begun, 3 ml blood were drawn every minute for 10 min, and also at 15 20 25 and 30 min after the beginning of the injection.

From every second blood sample 0 1 ml was taken immediately after collection for glucose determination by the method of Mark (15). The remainder of each blood-

sample was centrifuged within 10 min of collection and the plasma separated and frozen for later determination of insulin by a double antibody radioimmunoassay (Phadebas Insulin Test, Pharmacia, Sweden). The following test substances were administered

1) Cholecystokinin extracted from hog mucosa, containing 1 500 Ivy dog units/mg, obtained in sterile ampoules from the GIH Laboratory Karolinska Institutet, Stockholm, Sweden. Seventy-five Ivy dog units were given i v during 1 min to each subject.

2) Essentially pure cholecystokinin extracted from hog intestinal mucosa, containing 3 000 Ivy dog units/mg, supplied by Professor V Mutt the GIH Laboratory Karolinska Institutet, Stockholm. The extract was dissolved in 0.9 % NaCl and sterile filtered using Millipore filters which had been previously passed by a solution of protamine sulphate to saturate the protein-binding capacity of the filter The sterile solution of cholecystokinin was tested in vitro and it was found that no cholecystokinin activity had been lost in the sterile filtration procedure Seventy five Ivy dog units (25 μg) were given i. during 1 min to each subject.

3) The synthetic C-terminal octapeptide of cholecystokinin supplied by Dr M. A. Ondetti, the Squibb Institute for Medical Research New Brunswick, N.J USA, was dissolved in 0 9 % NaCl to a concentration of 0.33 μg/ml and sterile filtered as described above. By an in vitro assay using the guinea pig gallbladder it was ascertained that the biological activity was not reduced by the filtration procedure. Of this solution 7 ml were administered i.v to each subject during the first minute, followed by 3 ml/min until a total dose of 20 ml was reached, corresponding to '00 Ivy og units of cholecystokinin

The test substances were given in the order 3 2, 1 to four jects in the order 2, 3 1 to five and in the order 1 2, 3 to subject. A randomized order of administration of the three preparations was thus not adopted. Instead the inactive preparations were given first to all but one subject, as it was suspected that an active preparation might reduce the effect of preparations to follow within such a short period as 30 min (13).

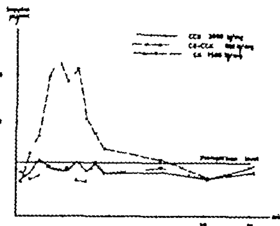


Fig 1 Mean plasma insulin levels in 10 subjects following i. administration of cholecystokinin extracts containing 1 500 and 3 000 Ivy dog units/mg, respectively and the synthetic C-terminal octapeptide of cholecystokinin (C8-CCK). Standard deviations are given in the text.

RESULTS

The administration of 75 Ivy dog units of partially purified cholecystokinin (1 500 Ivy dog units/mg) was followed by an increase in the plasma insulin concentration in all subjects The mean plasma insulin concentration in the group was significantly above the control level ($p<0.05$) from the 2nd to the 8th min after the beginning of the injection and then gradually returned to the control level (Fig. 1). A peak was seen between the 4th and 5th min after the beginning of the injection with a mean value of 33 μU/ml (Fig. 1).

Neither the synthetic C-terminal octapeptide of cholecystokinin nor the essentially pure preparation of the hormone (3 000 Ivy dog units/mg) produced any significant change in the plasma insulin level in the group (Fig. 1)

The standard deviation of the insulin concentration in the group was 4.2 and 5.3 μU/ml respectively for the two preinjection samplings between 3 1 and 7 5 μU/ml following the injection of the inactive substances and between 4 1 and 16.8 μU/ml following the injection of the partially purified preparation of cholecystokinin

None of the test substances caused any significant change in the blood glucose level Apart from slight abdominal discomfort for 1–2 min the subjects experienced no side-reactions to any of the test substances

DISCUSSION

The synthetic C-terminal octapeptide of cholecystokinin used in the present investigation possesses all the effects of the whole hormone molecule on gallbladder spincter of Oddi exocrine pancreas and small intestine (11 19). As the C-terminal octapeptide of cholecystokinin seems to be inactivated at a higher rate than extracted cholecystokinin (19) a larger dose in Ivy dog units was administered over a longer period This should guarantee a sufficient cholecystokinetic effect judged from an earlier investigation of this preparation (12) In spite of this the C-terminal octapeptide of cholecystokinin failed to release insulin in the subjects investigated To check the possibility that

an insulin-releasing effect might be bound to another part of the cholecystokinin molecule a preparation of extracted hormone considered to be practically 100% pure was in estigated Again there was no effect on the plasma insulin concentration

Our observations indicate that cholecystokinin-pancreozymin is of minor importance for the release of insulin in man. They are supported by an investigation by Young et al (21) who found that plasma pancreozymin levels rose only after a very large oral glucose load and the increase of pancreozymin then occurred later than that of insulin. The same highly purified cholecystokinin extract as was used by us has been given to rats by Dupré (7) and to man by Rabinovitch and Dupré (17) without any significant change in the plasma insulin level

Bertaccini et al. (2) found that coerulein produced an increased plasma insulin level in dogs. Coerulein is a decapeptide derived from frog skin but possessing a C-terminal structure identical to that of the C-terminal octapeptide of cholecystokinin except for one amino acid. The insulinotropic effect of this substance is not in line with the results of the present investigation but may be explained by species differences as Unger et al (20) found that a pancreozymin preparation of a purity comparable to the purest used in the present investigation released insulin and glucagon in dogs.

The insulinotropic activity in man following administration of cholecystokinin preparations of low purity which was found in many investigations (1 18), was verified in the present investigation which however provides evidence that the insulinotropic activity should be ascribed to the impurities rather than to the cholecystokinin content in these preparations

The identity of the insulin-releasing factor present in the impurities of some cholecystokinin preparations is not known. However it might be expected to belong to the glucagon-secretin group of intestinal hormones that has a well established insul notropic effect (9 14) Brown et al (4) have isolated substance named gastric inhibitory peptide from an impure preparation of cholecystokinin. The ga tric inhibitory peptide has structural similarities with glucagon (3). It has shown a high insulinotropic activity in preliminary experiments in man (10) The gastric inhibitory peptide should however be extensively removed from a cholecystokinin extract of the purity used in the present investigation (1 500 Ivy dog units/mg) (4) It is possible that the gastric inhibitory peptide is not the only insulin-releasing factor present in partially purified preparations of cholecystokinin.

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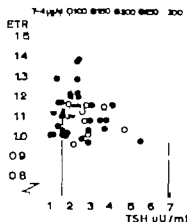


Fig 1 ETR and TSH in hypothyroid patients during euthyroidism caused by *l*-thyroxine replacement treatment. T-4=*l*-thyroxine dosage.

bound iodine, absolute free thyroxine and TSH determinations. The cause of hypothyroidism was surgical thyroidectomy in 16 cases, [131]Iodine treatment for thyrotoxicosis in 5 autoimmune thyroiditis without goitre in 22 and with goitre in 5 cases. The assessment of euthyroidism was done with the aid of a stable clinical normalization with no symptoms or signs of hyperthyroidism and with no need to change the daily dose of T-4 for at least 2 months. The clinical *lization was assessed according to recognized* l principles (6)

The TSH measurements were made by the Medix -tnical Laboratory by radioimmunoassay (4).

The ETR tests were made by the Res-O-Mat® ETR™ Test (Mallinckrodt Chemical Works, St. Louis, Missouri, USA) as described by the manufacturer as well as by various investigators (9 10 11 13 15). After denaturation of the proteins of the serum sample with alcohol, the separated patient thyroxine and an *aliquot of patient serum are introduced into buffered* solution of reference thyroxine-binding globulin labelled with [125]I-thyroxine The procedure displaces labelled thyroxine from the reference thyroxine-binding globulin and the labelled thyroxine is, in turn bound to free binding sites in the patient serum. The unbound labelled thyroxine is taken up by a resin strip and removed from the system. The amount of remaining labelled thyroxine is counted and the result is compared to that obtained with a similarly treated standard serum sample. The ETR is xpressed as counts per minute standard serum/counts per minute patient serum. The euthyroid range is 0.86–1 13

In our laboratory the radioactivity of the labelled reference thyroxine-binding globulin was checked for uniformity in every batch before use. The standard samples were always run in duplicate one at the beginning, one at the end of each series of determinations. The accuracy of the tests in multiple determinations from the same serum was ETR ±0 03

RESULTS

The results are presented in Table I and Fig. 1 All the ETR values are within or above the normal range of 0.86–1 13 and all the TSH values are within or below the normal range of 1.6–6.9 µU/ml The range of ETR values was 0.97–1.38 (mean 1.080) and of TSH values 1 1–5.5 µU/ml (mean 2.36) The range of T-4 dosage was 100–300 µg/day (mean 182).

DISCUSSION

In the evaluation of substitution treatment for hypothyroidism it is important that the patient s clinical improvement is the main guide-line to therapy It is necessary however that laboratory tests are used to supplement this guide-line In the choice of a laboratory test, not only its physiological implications but also its technical procedure must be considered It is generally agreed that in primary hypothyroidism a TSH decrease towards normal is the most reliable sign of commencing substitution. The TSH method is not, however suitable for routine work. Measurements of total thyroxine can be routinely used, but are not reliable when protein variations must be considered The same is true for the triiodothyronine-resin test. A combination of the two latter tests eliminates protein variation effects but is tedious because two separate test procedures have to be performed. The ETR, which is not disturbed by protein variations is done in a single test procedure and can be done in every laboratory where radioactivity measurements are made has definite advantages compared with the other tests.

Table I *Number of patients in the different ETR and TSH ranges and dosage groups*

+=above, —=below normal range N=normal

		l-thyroxine (µg/day)				
		100	150	200	250	300
ETR N TSH N	25	5	7	8	5	
ETR N TSH −	4	1	2		1	
ETR + TSH N	16	4	4	4	1	3
ETR + TSH −	3			1	1	1

In the present investigation the mean dose of T-4 is in accordance with recent observations in similar patients (12) The dosage of T-4 affects the laboratory values only slightly and the dose required to obtain euthyroidism varies as shown in Fig. 1 The higher doses of T-4 (250 or 300 μg/day) are, of course concentrated in the high ETR and normal or low TSH range On the other hand many medium doses of T-4 (200 μg/day) are seen in the normal ranges of TSH and ETR, and even in the high-normal range of TSH The low doses are more scattered This may be due to several reasons. Variations in absorption of T-4 from the gastrointestinal tract in different patients may play a role The occurrence of patients with low doses of T-4 in the high ETR range may favour this view On the other hand some patients obviously need high thyroxine concentrations to be kept euthyroid which is shown by the fact that ETR values up to nearly 1.4 may be necessary for clinical euthyroidism in some patients Similar observations have been made with other laboratory methods. The cause of this phenomenon may for instance, be variations in the deiodination of thyroxine as well as peripheral utilization of thyroid hormones It is obvious that T-4 treatment in hypothyroidism cannot be given in standardized dosage.

Both the TSH and the ETR are useful to exclude undertreatment with T-4 in primary hypothyroidism. They must however both be supplemented by a careful clinical evaluation if over dosage is to be avoided, as the range of values in both methods is wide for euthyroid patients. This clinical evaluation is necessary for the determination of the optimal dose and cannot at present be replaced by any available single laboratory method or combination of methods.

In the present material TSH determinations do not offer advantages compared with the ETR in the monitoring of the dose of T-4 The simpler and more rapid ETR measurement may thus well be used in such monitoring

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STUDIES OF URINARY BLADDER DYSFUNCTION IN AMYLOIDOSIS WITH POLYNEUROPATHY

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Abstract Eight male patients with amyloid polyneuropathy seven of whom had symptoms of dysfunction of the urinary bladder were studied by cystometry and micturition analysis and compared with eight male controls. Most of the patients with amyloidosis had an increased bladder capacity The need to micturate was reduced, and urine retention was common. In most of them detrusor function could not be demonstrated The urethral flow rate was usually diminished and the resistance to flow elevated. In some of the patients there was increased rigidity of the bladder wall.

In primary amyloidosis with polyneuropathy progressive sensory and motor disturbances are major manifestations of the disease Disturbances of motility in the gastrointestinal tract, postural hypotension, abnormal sweating, impotence and disturbances of micturition point to widespread involvement of the autonomic nervous system. Earlier descriptions of the disease mention incontinence regarded as an expression of sphincter dysfunction (4 5). As was shown in a previous study of patients in northern Sweden difficulty in emptying the bladder and infection of the urinary tract were frequent findings (3) Histopathological examination revealed amyloid deposits in the blood vessels and nerves of the bladder Amyloid deposits were also present in the detrusor musculature. The aim of the present investigation was to study the disturbance of micturition that develops in these patients by means of cystometry and micturition analysis To our knowledge there has been no previous detailed analysis of the disturbed micturition appearing in patents with this type of amyloidosis.

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MATERIAL

The material consisted of 8 male patients admitted to the Department of Medicine, University Hospital, Umeå. All had symptoms and signs of polyneuropathy most prominently in the lower extremities (Table I). The clinical signs pointed to involvement of the peripheral nerves and included sensory disturbances, muscle atrophy flaccid paralysis, and loss of reflexes The diagnosis of amyloidosis was confirmed by histological examination of biopsy material. The mean age of the patients at the time of investigation was 52 years (range 35-64). The cases are designated by capital letters as in earlier reports (1 2, 3) In the familial cases a numeral has been added The composition of the material is shown in Table I

The control group consisted of 8 men admitted to the Department of Medicine, University Hospital, Umeå. Data concerning the controls are given in Table II Their mean age was 46 years (range 37-58) None had symptoms or signs of peripheral neuropathy nor did they have any symptoms of disturbed micturition

METHODS

Clinical grading of neuropathy

The diagnostic criteria of polyneuropathy and the clinical grading of the peripheral neuropathy and of the gastrointestinal disturbances have previously been described (1 2) + = slight, ++ = moderate +++ = marked disturbances. Symptoms of bladder dysfunction impotence and rectal incontinence, if present, are not graded but merely indicated by +

Cystometry and micturition analysis

The technique sed is a modification of methods previously described (8, 12). Details of the procedure are given elsewhere (7). Two teflon catheters were inserted into the urinary bladder by suprapubic puncture. A third catheter was passed through one nostril and advanced into the stomach. The end of the catheter in the stomach was connected with thin-walled latex balloon, partially filled with air One of the bladder catheters was connected to an infusion apparatus con-

Table I *Data on 8 male cases of primary amyloidosis with polyneuropathy*

Case	Age at examination (y)	Duration of neuropathy (y)	Neuropathy[a] Arms	Neuropathy[a] Legs	Gastro-intestinal disturbances[a]	Rectal incontinence	Impotence	Symptoms of urinary dysfunction: Loss of sensation of bladder fullness	Delay in initiating voiding stream	Voiding by abdominal strain
A:6	64	8	++	+++	+	0	+	0	0	0
B.3	46	6	++	+++	+++	+	+	+	+	+
B.6	59	8	+	++	+++	+	+	+	+	+
C.2	35	6	++	+++	+++	+	+	0	+	0
F 1	62	6	+	++	+	0	+	0	+	0
J	38	2	+	++	0	0	+	0	+	0
K	57	4	+	++	++	+	0	0	+	0
L	57	7	++	+++	+++	+	+	+	+	+

+ = slight, ++ = moderate +++ = marked

taining physiological NaCl solution. The other bladder catheter and the stomach catheter were connected to a pressure-recording system consisting of pressure transducers with amplifiers and a multi-channel ink-flow recorder (EMT 34 EMT 31 Mingograph 81 Siemens-Elema, Stockholm) The registration system included subtraction circuit by which continuous subtraction of the intragastric pressure from the intravesical pressure could be obtained and thereby the contribution of straining to the intravesical pressure was deduced In some cases continuous subtraction was not performed the difference was then obtained by algebraic subtraction on the recorded pressure curves At mic tion the urine was collected in vessel resting on ... l frame furnished with a strain gauge receptor means of an amplifier and a derivation unit for ... ing the urine flow this receptor too was connected to the ink-flow recorder Both the urine volume and the rate of urine flow could be recorded.

The bladder was continuously filled with physiological NaCl solution at a rate of 50 ml/min while the intragastric and intravesical pressures and the difference between these pressures were simultaneously recorded. Every 50th ml instilled saline was noted on the re cording. The subject was allowed to void at increasing volumes up to and including that volume at which an urgent desire to void occurred. During micturition the various pressures the urine volume and urine flow were continuously recorded. In some cases the urine flow was obtained from the recorded volume curve After each voiding the residual rine was aspirated from the bladder through one of the catheters and was measured

Definitions

Bladder capacity (ml). The sum of the volume voided and the residual volume on the occasion of micturition

Table II. *Clinical data and findings of urinary bladder function in 8 male controls*

Case no	Age (y)	Clinical diagnosis	Bladder capacity[a] (ml)	Residual volume (ml)	Detrusor contraction pressure (mmHg)	Peak flow (ml/sec)	Urethral resistance (mmHg/(ml/sec)²)
1	53	Hypertonia	420	0	40	9 4	0.82
2	37	Hypertonia	500	0	35	13 9	0 33
3	52	Ulcerative colitis	445	20	33	17 4	0.20
4	43	Pulmonary fibrosis	520	60	26	10 1	0 49
5	42	Cardiomyopathy	350	0	20	24.3	0.08
6	37	Polycythemia	435	0	79	27 0	0.07
7	58	Splenomegaly	370	25	21	9.8	0.51
8	41	Pulmonary sarcoidosis	705	30	26	13.8	0.29
Mean			468	17	29	15 7	0.35
S D			112		7	6.7	0.25

Volume at urgent desire to void.

Weak voiding stream	In-conti-nence	Biopsy confirming amyloidosis
0	0	Skin, rectum
+	+	Skin, rectum
+	+	Skin rectum
+	0	Skin nerve
+	0	Skin rectum
+	0	Skin
+	0	Skin
+	+	Skin, rectum

at which the subject experienced an urgent desire to void.

Intravesical pressure (mmHg). Intravesical pressure in relation to 0-level through the superior border of the symphysis with the subject sitting or standing.

Premicturition pressure (mmHg). Intra esical pressure immediately prior to micturition—before the pressure rises due to contraction of the detrusor or pelvic floor or due to straining.

Detrusor contraction pressure (mmHg). Difference between the intravesical pressure when the subject does not strain, and the premicturition pressure.

Intraabdominal pressure (mmHg). Intragastric pressure in relation to atmospheric pressure

Peak flow (ml/sec). Maximal urine flow t mic turition.

Urethral resistance (mmHg/(ml/sec)²). Intravesical pressure divided by the square of the urine flow

RESULTS

Bladder capacity

The bladder capacities registered according to definition are given in Tables II and III The mean value for bladder capacity in the controls was 468±112 (S.D) ml For the patients with amy loidosis the mean value was 664±123 (S.D) ml Because of the impaired sensitivity discussed be low and because of the histopathological changes previously observed in the bladder wall in this type of patients (3) and the risk of overdisten-sion (13) Instillation was interrupted in some patients before an urgent desire to micturate had occurred In these cases the term "bladder capacity" corresponds to the maximum volume

Table III *Findings of urinary bladder function in 8 male patients with primary amyloidosis and poly neuropathy*

Case	Bladder capacity (ml)	Volume at desire to void (ml)		Volume at start of micturition/ residual volume (ml)	Detrusor contraction pressure (mmHg)	Peak flow (ml/sec)	Urethral resistance (mmHg/(ml/sec)²)
		Slight desire	Urgent desire				
A:6	545	N	No	390/170	26	6.4	1.37
				545/225	22	7 9	0.72
B.3	610*	400–500	No	430/165	0	3 7	5.30
				610/ 30	0	14.8	0.22
B:6	750	600–750	N	500/500	0	0	
				700/540	0	5.0	3.36
				750/645	0	3.0	7.56
C.2	700	No	No	340/340	0	0	
				600/525	0	2.0	20 00
				700/560	0	3 0	8.66
P 1	755	500	750	295/120	31	7 6	1 01
				395/ 60	35.5	7 1	1.26
				640/300	21.5	6.8	1 15
				755/215	11	8.0	0 75
J	750	500–750	No	370/370	0	0	
				790/625	0	5.6	1.91
K	435	250	425	150/ 10	0	5.8	0.66
				40/ 65	0	6.9	1.03
				435/ 70	0	6.4	0.43
L	770	620–770	No	665/615	0	4 7	1 78
				770/690	0	11 1	0.39
Mean	664					7.8*	_06*
S.D	123					3 7	2.87

Then incontinent Calculated on the highest, on the lowest individual values

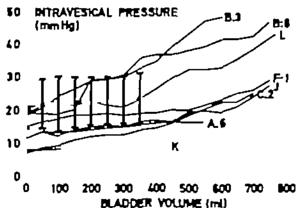


Fig 1 Intravesical pressure in relation to bladder volume. Observations from 8 controls (mean ±2 S.D thick lines) and from 8 patients with amyloidosis (thin lines)

of saline administered and is probably falsely low Nonetheless 5 patients with amyloidosis had a bladder capacity which exceeded the mean value for the control group by more than +2 S D The amyloidosis group had a significantly larger bladder volume than the controls ($0.01 > p > 0.001$ according to Student's t-test).

As the bladder fills there normally develops a feeling of fullness and the need to micturate By definition the value for bladder capacity in this dy corresponds to the volume at which an nt need to micturate occurred. The volumes hich this occurred in the controls are given in Table II The mean was 468±112 (S.D) ml When the bladder was filled two patients (F 1 and K) experienced an urgent need to void The volumes at this point were 750 ml and 425 ml respectively Four patients (B.3 B·6 J and L) had only a slight desire to empty the bladder even at volumes as large as 500 750 750 and 770 ml respectively Two patients reported no desire to void following instillation to 545 ml (A·6) and 700 ml (C.2) They reported only a slight feeling of fullness at these volumes. Four patients (B:6 F 1 J and L) experienced a desire to void urgent or slight only at volumes which exceeded the mean value for the control group by more than +2 S D

Little or no residual urine was measured in the controls (Table II) In the patients with amyloidosis on the other hand there was a more or less marked tendency to retention (Table III)

Intravesical pressure

The intravesical pressure at various volumes is shown in Fig. 1 In the control group the bladder pressure was initially 20.3±3.2 (S D) mmHg. With increasing volume the pressure in this group rose to 23 7±3.9 mmHg at 350 ml (the largest volume which all 8 controls attained). The pressure increase from 0 to 350 ml in this group was 3 4± 1.6 mmHg.

The patients with amyloidosis had resting blad

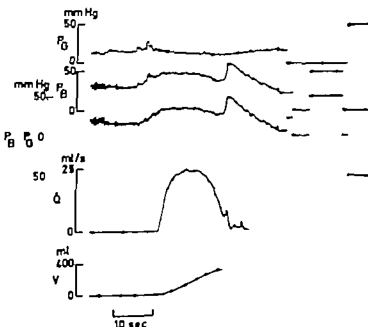


Fig 2 Simultaneous recordings during micturition without straining from a normal control subject. P_G=intragastric pressure P =intravesical pressure P_B–P = difference between intravesical and intragastric pressure. $\dot{Q}$=urine flow rate V = micturated urine volume

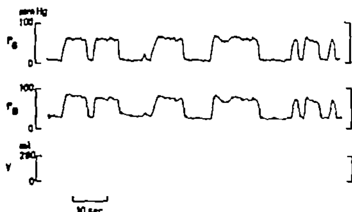


Fig. 3. Recordings during micturition from patient C.2 with insufficiency of the detrusor. Due to straining the intragastric and intravesical pressure curves are almost identical. Abbreviations as in Fig. 2.

der pressures which for small volumes were somewhat lower than the values for the controls. For an empty bladder the pressure was significantly lower in the patients than in the controls ($0.01 > p > 0.001$). The pressure, which was 13.7±4.9 (S.D.) mmHg initially, increased by 7.1±5.6 mmHg with a volume increase to 350 ml. Patients B.3, B.6 and C.2 had a pressure increase between 0 and 350 ml of 14.5, 17 and 7 mmHg, respectively. These values exceed the mean value for the controls by more than +2 S.D. No patient had a pressure increase that was less than the mean value for the controls −2 S.D. At volumes above 350 ml the intravesical pressure increased further in the patients (Fig. 1).

Detrusor contraction pressure

Values for this pressure, which corresponds to the increase of intravesical pressure due to contraction of the detrusor musculature in connection with micturition, are given in Tables II and III. All of the controls achieved peak flow during voiding by means of detrusor contraction without contribution from straining (Fig. 2).

All eight patients used abdominal pressure to micturate at the volumes investigated. In six patients (B.3, B.6, C.2, J, K and L) the increase in the intravesical pressure in connection with micturition was completely identical with the increase in intragastric pressure. This is illustrated in Fig. 3. In two patients (A.6 and F.1) the detrusor contraction pressure at peak flow was 26 and 35.5 mmHg, respectively.

Urine flow

The controls had values for maximal flow at micturition, peak flow, between 9.4 and 27.0 ml/sec, mean 15.7±6.7 (S.D.) (Table II). The peak flow in most of the patients was considerably lower (Table III). The mean value, calculated on the highest individual values, was 7.8±3.7 ml/sec. Patient B.3 had a peak flow value of 14.8 ml/sec and patient L a value of 11.1 ml/sec when the bladder volume at the start of micturition was 610 and 770 ml, respectively.

Urethral resistance

The resistance to flow in the urethra is shown in Tables II and III. The mean value for the controls was 0.35±0.25 (S.D.) mmHg/(ml/sec)². The mean value for the patients, calculated on the lowest individual values, was ...06+2.87 (S.D.). Patient B.3 had a resistance of 0.22 mmHg/(ml/sec)² at an initial bladder volume of 610 ml. Transiently patient L had a resistance of 0.39 mmHg/(ml/sec)² at a large initial bladder volume. At some time all patients had a urethral resistance that exceeded the mean value for the controls by more than +2 S.D.

Correlation between disturbances of micturition and clinical symptoms

As shown in Table I, all the patients except A.6 had experienced difficulties in connection with micturition. Seven reported difficulty in initiating micturition. They had also observed weakening of the urine stream. Three patients reported a need to strain in connection with micturition, while the remainder had not noted this. Two patients, A.6 and C..., experienced a need to void at bladder volumes of 545 and 700 ml, respectively. Prior to the study, however, they were not aware of any decrease of bladder sensitivity. Patients B.3, B.6 and L reported impairment of the sense of

bladder fullness. At the investigation they reported only a slight need to void at bladder volumes as large as 610 750 and 770 ml respectively

At comparison of the grades of other clinical manifestations (Table I) and the degrees of bladder disturbances no consistent agreement was revealed

DISCUSSION

The fact that most of the patients experienced slight or no desire to void in spite of the presence of a large volume of fluid and a high pressure in the bladder must be regarded as an expression of reduced sensitivity of the bladder wall The results of this study are in agreement with our previous findings on disturbances of micturition in patients with amyloid neuropathy (3) Histopathological examination of autopsy material revealed deposits of amyloid in the nerves of the bladder wall Such deposits affecting the nerve fibres can explain the reduced sensitivity demonstrated in the present study

Normally there are relatively large variations in the resting intravesical pressures between different individuals (12) Nonetheless our patients ppeared to have lower pressures at small bladder mes than did the controls

the other hand there are only small varia- in the resting pressure at different bladder olumes in one and the same person (12). This was the case in all of our controls. Among the patients however the pattern of individual pressure was different. The increase in the intravesical pressure that accompanied increase in the bladder content was strikingly large in most of the patients This may be attributable to increased rigidity of the bladder wall due to amyloid deposits in the detrusor musculature Such deposits, which have been observed in autopsy material are described elsewhere (3). Histopathological examination showed that, in place the smooth muscle fibres in the detrusor were completely replaced by amyloid This rigidity of the bladder wall is probably analogous to that which occurs in the myocardium in cardiac amyloidosis (14)

In normal subjects the perivesical pressure increases during filling of the bladder (7) Whether the perivesical tissue has an increased rigidity in these patients with amyloidosis and in what way this may influence the intravesical pressure rise is an open question.

At the time of micturition the intravesical pressure in the controls increased with very little contribution from abdominal pressure The increase in pressure which occurred due to detrusor contraction agrees with the values measured in other comparable materials (11 12). All the patients on the other hand needed to strain to micturate at the bladder volumes investigated In only two of the eight patients could the increase in pressure be partly attributed to detrusor contraction This impairment of the detrusor function is probably due mainly to amyloid infiltration of the nerves in the bladder The extent to which amyloid deposits in the musculature decrease the contractility of the detrusor cannot be stated

Generally the maximal urine flow at micturition was lower and the resistance to flow in the urethra was greater in the patients than in the controls This can be explained by the impairment of the detrusor function Normally the detrusor not only brings about an increase in the intravesical pressure but also shortens and widens the proximal part of the urethra (8) The resistance is thus decreased and the urine can pass the "inner urethral orifice" At increased bladder volumes and increased intravesical pressures there was some lowering of the resistance to flow in most of the patients This can be explained by an increased tension in the bladder wall associated with increased volume leading to some although incomplete widening of the proximal urethra and thereby diminishing urethral resistance The impairment of the detrusor function is an important factor in the disturbance of the emptying mechanism. Whether alteration in the relaxation capacity of the striated musculature of the urethra plays a role cannot be stated

The impairment of bladder emptying, demonstrated in these patients with amyloidosis may cause chronic retention and predispose to infection of the urinary tract with risk for pyelonephritis (3)

The disturbance of micturition that occurs in this type of amyloidosis is in some respects similar to that observed in diabetes mellitus with polyneuropathy In the latter there is also an increased bladder capacity and a tendency to urine

retention (9). Defective opening of the internal meatus of the urethra has been described in connection with roentgenological analysis of bladder emptying and a possible connection with impaired detrusor function has been discussed (6). Direct measurements of detrusor function and of urine flow however do not seem to have been performed. The bladder in diabetics is reported to be hypotonic compared with that in normals (10 15). In this respect the bladder in amyloidosis with polyneuropathy often seems to differ markedly from that in diabetes mellitus

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LUPUS-LIKE SYNDROME ASSOCIATED WITH PULMONARY REACTION TO NITROFURANTOIN

Report of Three Cases

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Abstract. A systemic lupus erythematosus-like syndrome developed simultaneously with pulmonary reactions of the chronic type in three female patients after treatment with nitrofurantoin for 12, 27 and 38 months, respectively The syndrome was characterized by elevated ESR, polyclonal hypergammaglobulinaemia, the presence of IgG antinuclear antibodies and positive latex-fixation test. Two patients had severe arthralgia and one of them peripheral lymphadenopathy Pleural effusion and chronic active hepatitis were present in the third patient, in whom interstitial cystitis also developed All signs and ymptoms of the lupus-like syndrome disappeared, without corticosteroid or other medication, when nitrofurantoin was omitted. The diminution of pulmonary infiltrates and the reversal of interstitial cystitis also ppeared to be directly related to cessation of nitrofurantoin therapy Our findings indicate that long-term medication with nitrofurantoin may cause in addition to pulmonary changes, a simultaneous lupus-like syndrome Our data also raise the possibility that interstitial cystitis may occur as an adverse reaction to nitrofurantoin therapy

Since pulmonary reactions to nitrofurantoin were first reported in 1962 (7) an acute and a chronic type of reaction have been documented The acute reaction, reported with increasing frequency occurs after readministration of nitrofurantoin to previously sensitized patients is regarded as an immunological type III reaction and is characterized clinically by sudden fever dyspnoea, eosinophilia and pulmonary infiltrates that resemble pneumonia or pulmonary oedema (3 6 9). The chronic reaction develops after administration of nitrofurantoin for several months or years and is marked by radiographically demonstrable fibrosis with or without interstitial pneumonitis Neither fever nor prominent eosinophilia are associated with the chronic reaction. With the exception of two patients reported by Back et (4), no distinct immunological abnormalities have been identified in patients with the chronic reaction (1 5 8 11 13 16–21).

We report here on three patients whose reactions to nitrofurantoin were of the chronic pulmonary type and in whom a syndrome typical of systemic lupus erythematosus (SLE) developed simultaneously with the pulmonary manifestations. Interstitial cystitis also developed in one of these patients

CASE REPORTS

Case I

Female born in 1892. Her mother suffered from rheumatoid arthritis. At the age of 50 the patient was afflicted with arthralgia and was, on this account, xamined several times during the next ten years. Neither radiographical bone surveys nor serological tests provided any vidence of rheumatoid arthritis.

In March 1969 the patient was admitted to the hospital because of nausea, dyspnoea, dizziness and peripheral oedema. Congestive heart failure was diagnosed and was treated with digitalis and diuretics. Mild diabetes was found and was treated with dietary regimen. Urine culture yielded an abundant growth of Proteus mirabilis. Renal function, ESR and peripheral blood picture were normal. An pyelography revealed no abnormalities Neither rheumatoid factor nor antinuclear antibodies were demonstrable The Coombs test was negative. Serum protein was 7 6 g/100 ml γ-globulin 1.25 g/100 ml

Initial treatment of the rinary tract infection with sulphonamide was discontinued after eight days when urticaria developed On April 14 1969 treatment with nitrofurantoin, 150 mg daily was started. Four eek later the patient was discharged in good condition, continuing treatment with nitrofurantoin digitalis and diuretics. While in hospital her ESR had sen from normal values to 45 and then to 65 mm/h without explanation.

Table I *Clinical and laboratory data of three female patients with chronic pulmonary reaction and simultaneous lupus-like syndrome induced by nitrofurantoin*

Pat. no	Age (y)	Nitrofurantoin therapy: Daily dose (mg)	Duration (mo)	Clinical symptoms and signs	Abnormal laboratory test results	Course after withdrawal of nitrofurantoin without other treatment
1	79	150 100 50	5 8 14	Arthralgia, lymphadenopathy pulmonary infiltrates and fibrosis	ESR 12–1.5 hypergammaglobulinaemia antinuclear antibodies total titre 1 320 IgG titre 1 320 IgM titre 1 10 latex+ direct Coombs test+ thyroglobulin antibodies titre 1 2.5 mill	Regression of pulmonary infiltrates. Disappearance of the enlargement of lymph nodes and arthralgia. Results of laboratory tests returned to normal
2	61	100	12	Arthralgia, pulmonary infiltrates	ESR 16–80 hypergammaglobulinaemia, antinuclear antibodies total titre 1 1 280, IgG titre 1 1 280 IgM titre 1 20, latex+	Chest X-ray and results of laboratory tests returned to normal
3	75	150 100	17 21	Fever pulmonary infiltrates and slight fibrosis, pleural effusion, chronic active hepatitis (?)	ESR 25–112, hypergammaglobulinaemia, antinuclear antibodies total titre 1 1 000, IgG titre 1 100 IgM titre 1 10 latex+++ direct Coombs test+ smooth muscle antibodies+ 1 100	Regression of pulmonary infiltrates. Disappearance of pleural effusion. Improvement in laboratory test results

At follow-up examinations sterile pyuria was repeated finding, but urine cultures yielded no significant bacteriuria. The ESR continued to rise (73–84 mm/h). On t. 19 1969 the dosage of nitrofurantoin was reduced to mg daily and six weeks later was omitted altogether use of vomiting. The patient s subjective condition *proved rapidly after withdrawal of nitrofurantoin and her* ESR began to drop (50–37–18 mm/h) the lowest value being recorded four months later N trofurantoin, 100 mg daily was then given again because of pyuria and was continued until Dec. 1971 During this 22-month period the ESR gradually rose from 18 to 125 mm/h serum protein from 8 1 to 9 4 g/100 ml γ-globulin from 2.2 to 3 4 g/100 ml. A chest X-ray taken in Aug. 1970 revealed her lungs to be clear

When an X-ray taken in Aug. 1971 disclosed patchy pulmonary infiltrates, tuberculosis was suspected Repeated TB cultures of sputum were negative The patient was readmitted to the hospital in Nov 1971 when a follow up X-ray examination revealed increasing fibrosis of the lungs

On admission the patient s only complaint was increased pain in various joints. She had no dyspnoea at rest. BP was 160/95 Skin and heart auscultation and thyroid, renal and liver function tests showed normal values. Neither liver nor spleen was palpable but the cervical, axillary and submandibulary lymph nodes were enlarged (5–15 mm). ESR was 102 mm/h. The peripheral blood picture was normal except for light eosinophilia (8.5–9.5% of 4700 WBC) Urinary tract examinations revealed a benign papilloma of the bladder that was subsequently electrocoagulated. Hypergammaglobulinaemia was still present (3 1 g/100 ml) *and consisted mainly of an increase in IgG Immunological* tests disclosed the abnormalities listed in Table I. The Waaler Rose test, however was negative LE cells were not found.

Because the pulmonary changes were suspected of being *caused by nitrofurantoin, the drug was withdrawn. One* month later moderate improvement was already noticeable and since then the chest X-ray has remained unchanged during a follow-up period of 2.5 years. Enlargement of the lymph nodes disappeared within tw months of discontinuing nitrofurantoin therapy and the results of laboratory tests have gradually returned to normal values. The ESR fell from 102 to 34 mm/h the slight eosinophilia disappeared, serum protein and γ-globulin levels returned to normal the titre of antinuclear antibodies decreased to *1 40 of thyroglobulin antibodies to 1.25 000* erythrocyte antibodies were no longer demonstrable and the latex fixation test is now negative. The patient is currently in fairly good health and is being treated with digitalis and diuretics. The arthralgia persists with the same severity as before administration of nitrofurantoin.

Case 2

Female born in 1911 Her mother and two sisters suffered from rheumatoid arthritis. The patient was in good health until 1965 when she suffered moderate congestive heart failure. Since 1967 recurrent urinary tract infections have been treated with various drugs. Hypersensitivity to sulphonamides was noted. In March 1971 she was examined in the outpatient department of local hospital

because of acute febrile pyelonephritis. A urine culture yielded growth of Escherichia coli. Treatment with nitrofurantoin, 100 mg daily was started and subsequently supervised by a local physician. During the follow-up period in 1971 urine cultures showed no bacterial growth. The ESR however gradually rose from normal values to 66 mm/h without explanation.

In Dec. 1971 during visit to the Canary Islands, joint pain, especially in the large joints, developed. No explanation for this pain was found at examination after her return to Finland. Rheumatoid arthritis and infectious arthritis ere excluded.

Three months later the patient ESR was 75 mm/h and she was admitted to hospital for further examinations. She was then af bole and her subjective symptoms were pain in joints and dyspnoea. Physical examination revealed BP of 150/80, a systolic murmur of first degree over the cardiac apex, normal skin and joint state and no enlargement of lymph nodes, spleen or live Crepitating rales ove the *bases of both lungs were however audible and a chest X-ray* showed bilateral interstitial infiltrates in the lower lobes of both lungs. The ESR was 80 mm/h blood picture was normal. Urinalysis revealed only slight pyuria. Renal function was normal and ormal values were obtained for blood sugar and liver enzyme tests. Serum protein was 8.2 g/100 ml with moderate increase in the γ-globulin fraction (1.8–2.0 g/100 ml). Immunoelectrophoretic investigations showed slight increase in the IgA band. Antinuclear antibodies of the IgG type were demonstrable (Table I) the latex-fixation test was positive but the Waaler-Rose titre normal LE cell tests were not done.

Nitrofurantoin was suspected of causing the pulmonary changes and the treatment was stopped. Two weeks later moderate regression of the pulmonary infiltrates was observed, and five months later the chest X-ray was normal. Simultaneously the ESR dropped to 25 mm/h, the latex-fixation test was negative and the titre of antinuclear antibodies decreased to 1 320. One year later (in May 1973) this titre was only 1 20.

The patient is currently in good health. She had neither dyspnoea nor joint pain. Her congestive heart failure is continuously treated with digitalis and diuretics. Urinary sediment and enal function have been normal during the observation period.

Case 3

Female, born in 1899. The patient was in good health until the summer of 1970 when she was troubled with dysuria. A urine culture yielded growth of E. coli and she was treated by local physician, first with chloramphenicol and later bh nitrofurantoin, 150 mg daily. In Nov. 1970 and again two months later the ESR was 44 mm/h and the urinary sediment still showed haematuria, pyuria and Gram-negative bacteria.

In Feb. 1971 she was seen as outpatient in our hospital for further investigation. She was found to be in good health but complained of dysuria and periodic pai over the kidneys. The pulse was 90 and BP 175/105 peripheral blood picture, renal function, chest X-ray and results of heart and lung auscultations were normal. Peripheral lymph nodes, liver and spleen were not palpable. Tests for rheumatoid factor and antinuclear antibodies were negative. I pyelography revealed morphologically normal kidneys. The ESR had risen to 64 mm/h. The urinary sediment still contained red and whit cell in abundanc but no bacteria. Urinary TB cultures were negative. Treatment with nitrofurantoin was continued.

A cystoscopic examination, performed in June 1971 revealed reddening area near the left ureteral orifice. Th bladder volume was 250 ml and biopsy disclosed inflammatory changes. When the bladder was reinspected on month later gross and microscopical findings were consistent with interstitial cystitis.

In Sept. 1971 the urinary sediment still showed haematuria and pyuria. The results of third cystoscopic examination were the same as the earlier tw bladder volume was 300 ml. In Jan. 1972 biopsy of the bladde still provided the patho-anatomical diagnosis of interst ial cystitis.

In March 1972 the patient had paroxysmal entricular tachycardia. This episode was promptly corrected with lidocain. *Myocardial infarction was excluded. Treatmen* with digoxin, furosemide and alprenolol was started. The nitrofurantoin therapy that had been maintained for 17 months was stopped. Urinary sediment showed slight haematuria but was otherwise normal.

One month later urinalysis revealed red cells in abundance but a urine culture yielded only insignificant growth of enterococci. Nitrofurantoin, 100 mg daily as given again as precaution and was continued until Jan. 1974. During this period the ESR rose from 30 to 107 mm/h.

In Dec. 1973 the patient fell acutely ill th fever and dyspnoea. When fever persisted for two eeks in spite of treatment with antibiotics she was admitted to the hospital. On admission she was severely fatigued. Nitrofurantoin was withdrawn. The temperature was 39.0–39.9°C but returned to normal without antibiotics on the third day in hospital. The pulse rate was 70 respiration 4 and BP 130/70. There were no signs of congestive heart failure. The liver was not enlarged and neither spleen nor peripheral lymph nodes were palpable. The blood picture was normal. Urinalysis and renal function tests showed normal values. Rales and rhonchi were however audible over both lung bases. A chest X-ray showed bilateral infiltrates, most prominently in the middle and lower lobes of the right lung. Pleural effusion was seen on the right side. Serum protein was 7.9 g/100 ml but the γ-globulin fraction was polyclonally increased (3.3 g/100 ml). The latex-fixation test was strongly positive but the Waaler Rose titre only 1 64. Antinuclear antibodies, smooth muscle antibodies and red cell antibodies were also detected (Table I). The serum bilirubin was normal but five enzyme tests disclosed pathological picture. SGOT was 142 U/l at 25°C SGPT 76 U/l at 25°C 5LDH 315 U/l and alkaline phosphatase 463 U/l (normal below 48 U/l).

Results of liver enzyme tests gradually returned to normal during the 4-month hospital stay. J st before these results were within normal limits, liver scan was taken and found to be normal. A cytological fine-needle aspiration biopsy revealed that hepatocytes were normal but that mononuclear cells, mostly lymphocytes but few plasma cells, were present in increased numbers. Chronic active hepatitis was suspected but histological needle biopsy of the liver was normal.

During the follow-up period, in which the patient was given neither nitrofurantoin nor other antibacterial drugs a regression of the pulmonary symptoms occurred and the chest X-ray is now almost normal The ESR has dropped to 50 mm/h Immunofluorescent antinuclear antibodies are no longer demonstrable the direct Coombs test is negative and liver enzyme tests are normal. The latex-fixation test is still positive (++ 1 64) the Waaler Rose titre 1 64 The patient has no pain in or swelling of joints, no skin lesions no enlargement of peripheral lymph nodes. She has no dysuria and a cystoscopic examination in May 1974 revealed quite normal bladder with normal volume (400 ml)

DISCUSSION

Since it was introduced in 1953 nitrofurantoin has been used extensively in the treatment of urinary tract infections The drug is known to have toxic and allergic side-effects The most serious toxic side effect is dose-related polyneuropathy especially prone to occur in patients with impaired renal function treated with normal doses of the drug. Haemolytic anaemia megaloblastic anaemia, aplastic anaemia and agranulocytosis are rare toxic side effects. The most serious side-effect of the allergic type is an acute pulmonary reaction characterized by sudden fever dyspnoea and cough In such reactions pleural effusion blood eosinophilia and infiltrates that resemble pulmonary oedema or pneumonia are common (3 6, 9)

In 1966 Sollaccio et al (19) drew attention to a case subacute pulmonary infiltrates caused by nitrontoin Their patient had taken nitrofurantoin ...ily for one year and symptoms had been present for at least one month Clearly chronic pulmonary reactions in five patients, the result of treatment with nitrofurantoin were reported in 1968 by Rosenow et al (17) Since then similar cases have been documented (1 5 8 11 13 16, 18 20 21)

The three patients we report on had all been treated for urinary tract infections with nitrofurantoin for long periods—1 27 and 38 months respectively Pulmonary infiltrates developed in all three Partly fibrotic lesions developed in the lungs of two of the patients of whom one also had pleural effusions. Slight blood eosinophilia was noted in only one patient. After cessation of nitrofurantoin therapy a gradual improvement of the pulmonary manifestations took place without need for simultaneous corticosteroid or other treatment. In all three cases the start of improvement was closely related to the withdrawal of nitrofurantoin suggesting a direct relationship between the drug and the pulmonary manifestations Although histological evidence of the pulmonary lesions we describe is lacking it seems obvious that these pulmonary reactions are of the same type as those reported previously to have been the result of nitrofurantoin therapy

Interesting concomitant findings are the immunological abnormalities that developed simultaneously with the pulmonary reactions. These included a markedly elevated ESR polyclonal hypergammaglobulinaemia a positive latex-fixation test with either a negative or low Waaler-Rose titre as well as the presence of IgG antinuclear antibodies. These alterations are typical of SLE which in 3–12% of cases has been shown to be drug-activated" (10) The arthralgia of two patients the peripheral lymphadenopathy of one of them and the possible chronic active hepatitis of the third also conform with the lupus-like syndrome The findings are very similar to those observed in two patients by Bäck et al (4)

Toxic hepatic reactions to nitrofurantoin accompanied by non-haemolytic jaundice have occasionally been reported (12) but involvement of the liver without jaundice has been documented in only three patients (2, 4). In one of the latter (2) a hepatic reaction with elevated SGOT and alkaline phosphatase levels developed following two weeks therapy with nitrofurantoin The patient also had myalgia arthralgia an elevated ESR and a diffuse pruritic maculopapular rash on both legs. It seems obvious that the nature of the liver damage without jaundice of this patient is different from that of our case 3 in which hepatic involvement represents not toxic damage but more likely a manifestation of a simultaneously occurring lupus-like syndrome The infiltration of the liver by mononuclear cells and the presence of smooth muscle antibodies also suggest an immunological rather than a toxic reaction of the liver

Syndromes indistinguishable from SLE have been reported following the administration of a large variety of drugs These include hydralazine procainamide mephenytoin diphenylhydantoin tri methadione phenobarbital isoniazide penicillin tetracycline sulphonamides and others (14) Nitrofurantoin can now be added to drugs causing a lupus-like syndrome

As has been typical in lupus-like syndromes caused by other drugs the immunological abnormalities disappeared spontaneously after with-

drawal of nitrofurantoin. The fact that family members of at least two of our patients had a history of other connective tissue disease would support the hypothesis that 'drug-activated SLE develops only in persons who have a specific predisposition to the disease

The biopsy-verified interstitial cystitis of our case 3 is also of special interest. It was diagnosed after one year s treatment with nitrofurantoin and was detectable as long as the treatment was continued Five months after withdrawal of nitrofurantoin a cystoscopic examination revealed a normal urinary bladder Inasmuch as interstitial cystitis is generally regarded as a chronic disease that terminates in irreversible fibrosis the course of the illness of this patient has thus so far been unusual. Interstitial cystitis is moreover considered to be an immunological disease (15) Its development therefore in a patient who also develops a lupus-like syndrome secondary to drug therapy is not unexpected and would conform with the postulate of a genetically determined predisposition. However the possibility that the interstitial cystitis in this case was in fact a reaction to the nitrofurantoin therapy cannot be excluded either especially in view of the reversibility of the bladder manifestations The interstitial cystitis may in this patient represent an untoward reaction similar to the pulmonary reaction which we believe to be an adverse reaction to nitrofurantoin.

Nitrofurantoin is a widely used and usually well tolerated drug. Although the chronic pulmonary reaction and concomitant lupus-like syndrome that we describe are rare complications we believe that the calling of attention to such possible adverse reactions might help us to avoid such serious complications.

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COMPLEMENT SYSTEM STUDIES IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

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Abstract Complement system involvement has been studied in 16 patients with systemic lupus erythematosus (SLE). Circulating conversion products of C3 were observed in 4 cases. Low mean values of C4 and C3 were found, while C3 proactivator (properdin factor B) levels were low in only a few of the patients. The levels of C4 C3 and C3 proactivator were not lower in the 4 patients in whom C3 conversion products could be demonstrated than in the others. It is concluded that the low complement values found in SLE may be caused mainly by deficient synthesis. Signs of complement activation are in this patient material demonstrated early in the disease, and chiefly in patients not receiving immunosuppressive therapy

The main manifestations of systemic lupus erythematosus (SLE) are probably caused by circulating complexes of nuclear antigen and antinuclear antibodies (2, 7) Considerable interest has therefore been focused on the chief effector pathway of humoral immunity the complement system in SLE Renal and dermal depositions of C4 C3 and properdin have been taken as evidence of the pathogenetic significance of complement in this disease (16) Profound changes in total haemolytic complement and in levels and activity of individual components of the classic and alternate pathways of complement activation can be demonstrated (4 5 6 8 11 13 14) The finding of low complement activity and low levels of individual factors in SLE has usually been regarded as firm evidence of in vivo activation Studies in recent years have thrown doubts on this concept by showing that low C3 levels in disease could be caused mainly by deficient synthesis of the protein (1) Conflicting results have emerged from turnover studies using radioactively labelled C3 and the pathogenetic significance of the complement system in SLE remains unclear (6, 17)

The present studies were performed with the aim of elucidating further the mechanism(s) behind the low complement levels often found in SLE

MATERIAL AND METHODS

Subjects

The patient material includes 16 individuals (11 females). The diagnosis was in each case based on published criteria (3). Most patients were studied on one occasion only six on 2–4 occasions during 2 year period.

Blood samples

Venous blood was collected from both controls and patients and left to coagulate without additives at 20°C Within 2 hours serum was separated from the blood cell and placed in a freezer at −75°C until the tests were performed 1–10 days later

Crossed immunoelectrophoresis

Crossed immunoelectrophoresis was performed according to Laurell (9) with slight modifications. The first separation was performed as high voltage (20 V/cm) agarose gel electrophoresis in barbital buffer for 2 1/2 hours as earlier described (18). The gel strip containing the separated serum proteins was cut off and placed in slot in an anti-C3-containing agarose gel. The second electrophoretic run was then performed at right angles to the initial separation and under identical conditions (Fig. 1)

It has been shown by Laurell and Lundh (10), and confirmed in this laboratory that when the blood samples are handled in the manner described above and the serum proteins are separated by high voltage electrophoresis in agarose gel, only traces of C3 conversion products can be found in normal serum In this laboratory 50 serum samples from 35 normal have been tested under these conditions, and no significant amounts of C3 conversion products have been found in any of these sera. EDTA plasma is less reliable in experiments of this kind, possibly because of precipitation of fibrin in the calcium-containing gel (Teisberg, unpublished observations)

Comparison of levels of C3 degradation products can be made between patients, and in one and the same patient on different occasions by planimetry I this study the

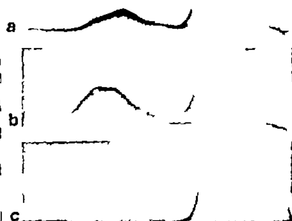


Fig 1 Crossed immunoelectrophoresis of sera from SLE patients with circulating conversion products of C3 (*a* *b*) and normal control serum examined by the same method (*c*) Anode is to the left in the first electrophoretic separation and on the top in the second.

sera have mainly been classified as positive or negative with regard to content of C3 conversion products.

Immunochemical determinations

C4 C3 and C3 proactivator (properdin factor B) determinations were performed by the radial immunodiffusion method according to Mancini et al. (12)

Antisera

Anti-C3 was produced in rabbits immunized with human C3 isolated by preparative electrophoresis on agarose gel The antiserum contained two weak contaminating antibodies. The antiserum used in radial immunodiffusion experiments was therefore absorbed with a patient serum deficient in C3 Specific anti-C4 was provided by Dr C Alper Boston Anti-C3 proactivator was obtained from Behringwerke Marburg/Lahn

Reference serum

Standard human serum (Behringwerk) was used as reference serum for the determination of C3 and C4 in the radial immunodiffusion experiments

RESULTS

Relevant clinical data concerning the patient material are given in Table I

C3 conversion products were demonstrated on at least one occasion in 4 of the 16 patients with SLE (Table II) Of the 6 patients tested on several occasions 5 were consistently negative One patient (no 8) had initially circulating conversion products of C3 Later on after initiation of prednisone and azathioprine treatment, she became negative and has remained negative and has been in complete clinical remission for one year Three of the 4 positive patients did not receive immunosuppressive therapy at the time of the study while 9 of the 12 negative patients were on prednisone alone or combined with azathioprine

C4 C3 and C3 proactivator levels are given in Table II It can be seen that mean levels of both C4 and C3 were lower than normal mean Only two patients had C4 levels and one patient had a C3 level above the normal mean. In 4 patients extremely low levels of both componenets were found There is good correlation between C4 and C3 levels in the individual patients ($r = 0\ 9049$ $p < 0\ 001$) The mean C3 procativator level in the patient group did not differ significantly from that found in normals. The patients with very low C4 and C3 levels, however had also lower than normal C3 proactivator levels.

No differences in complement component levels were found between the groups with and without C3 conversion products

Disease activity as judged by clinical symptoms and signs was present in all 4 patients with C3 conversion products but also in 5 of the other 12 patients. Lower mean levels of C4 and C3 were found in the patients with disease activity than in those with non-active SLE but the differences were not statistically significant.

DISCUSSION

It is generally accepted that total haemolytic complement, biological activity of severel of the factors and the levels of these factors measured immunochemically are often decreased in SLE patients In this study low C4 and C3 levels have been found and the decrease is of approximately the same degree for both factors Low C3 proactivator levels were found less constantly Others have reported that low C4 levels may be seen even more often than low C3 levels (8 15) C1q and C2 are usually depressed while one study reports normal C5 levels (8). Properdin levels are low in patients with high disease activity (13) This shows that the complement abnormalities in this disease affect both the classic and the alternate or properdin pathways

Table I *Clinical and laboratory data*

For patients studied more than once the first set of laboratory values is given

Case no	Sex	Age (y)	Duration (y)	ESR	ANF	LE cells	IgG	IgM	IgA	Treatment when first seen	Organ manifestation
1	♀	23	10	6	+	+	1 200	130	70	Prednisone	Nephritis mucocutaneous lesions, serositis
2	♀	33	13	26	+	+	2 750	80	40	Prednisone azathioprine	Nephritis arthritis, mucocutaneous lesions
3	♀	18	4	11	+	+	2 050	140	300	None	Arthritis, vasculitis, lymphadenopathy
4	♀	34	3	38	+	+	1 800	120	180	Prednisone, azathioprine	Nephritis, arthritis, pancytopaenia
5	♀	26	6	29	+	+	1 300	110	310	None	Nephritis, arthritis, pancytopaenia, mucocutaneous lesions
6	♀	33	13	85	+	+	2 300	200	190	Prednisone azathioprine	Nephritis, vasculitis, mucocutaneous lesions
7	♀	26	6	11	+	+	550	140	60	Prednisone	Nephritis vasculitis, mucocutaneous lesions, CNS manifestations
8	♀	28	2/12	31	+	+	2 000	620	220	None	Nephritis, arthritis, mucocutaneous lesions
9	♀	27	4	11	+	–	1 650	50	40	Prednisone azathioprine	Arthritis, mucocutaneous lesions, CNS manifestations
10	♀	44	6	32	+	+	100	200	180	Prednisone	Nephritis arthritis, mucocutaneous lesions
11	♂	34	5	105	+	+	1 800	190	670	Prednisone	Vasculitis mucocutaneous lesions, CNS manifestations
12	♀	32	10	60	+	+	2 650	150	250	Prednisone, azathioprine	Nephritis, arthritis, mucocutaneous lesions, thyroiditis, liver manifestations
13	♂	38	4	13	+	+	2 750	90	70	None	Nephritis, CNS manifestations, liver manifestations
14	♂	34	6	30	–	+	2 500	340	530	None	Arthritis, mucocutaneous lesions, vasculitis
15	♂	29	1	41	+	–	2 200	280	320	Prednisone	Nephritis, arthritis, vasculitis
16	♂	52	1	105	+	+	3 350	120	140	None	Nephritis, arthritis mucocutaneous lesions, vasculitis carditis

Alper and Rosen (1) were the first to show by turnover studies of radioactively labelled C3 that low levels of this factor in glomerulonephritis could be caused by decreased synthesis and not necessarily by in vivo activation. Hunsicker et al (6) in their study of a large group of SLE patients concluded that the low C3 levels were caused mainly by complement activation. In contradiction to this the turnover study of Sliwinski and Zvaifler (17) showed that decreased synthesis was the chief reason for the low C3 content in their SLE patient sera

In the present patient group in vivo activation of C3 seems to take place at a significant rate in only a small proportion. Even the sera containing C3 degradation products contain rather low levels compared to what can be found for instance in some patients with glomerulonephritis and chronic active hepatitis (19 20) and some of these latter patients may even have normal C3 levels. One of the present patients activating C3 had a C3 level at the normal mean and of the three patients with very low C4 and C3 levels (nos 2 15 16) C3 activation could be demonstrated in only one. The present results thus support the findings reported by Sliwinski and Zvaifler (17) and it may be concluded that in this group of patients low complement factor levels cannot be explained by in vivo activation alone. It is possible however that the results reported by Hunsicker et al (6) are caused by the selection of patients in an early stage of the disease

In the present patient material a moderate degree of in vivo activation of C3 is found in some individuals generally said in those with relatively short disease duration and those who have not been treated with immunosuppressive drugs. It seems that the immunological activity burns out to some extent after a few years, even if the disease process propagates in some organ system. The seemingly low activity in the humoral immune system may also be the result of intense immunosuppressive therapy in some patients as apparently is the case in our patient 8. It may be concluded that in the present

Table II Results of the screening for circulating conversion products of C3 and of the immunochemical determinations of C4 C3 and C3 proactivator levels in serum

The evaluation of disease activity is based on clinical symptoms and signs. For patients studied more than once the first set of results is given

Case no.	Disease activity	C3 conversion products	C4 (mg/100 ml)	C3 (mg/100 ml)	C3 proactivator (% of normal mean)
1	–	–	18.8	57	106
2	+	–	0.8	13	60
3	–	–	18.8	55	99
4	–	–	19.0	56	104
5	–	–	13.1	38	82
6	+	–	17.4	45	74
7	+	–	19.8	46	100
8	+	+	16.2	36	104
9	+	+	21.2	49	111
10	–	–	22.0	54	111
11	+	–	25.8	60	114
12	–	–	14.6	38	73
13	+	+	24.3	85	136
14	–	–	26.3	98	158
15	+	–	0.9	11	65
16	+	+	1.1	9	42
Mean for patient			16.3	46.9	96.2
Mean for normals			23.4	97.0	100
Normal range (mean±2 S.D.)			12.8–34.0	64–130	68–132

ent group the possible pathogenetic significance complement activation is mainly limited to the early phase of the disease

The reason for the low complement factor levels found in so many SLE patients remains unknown. A negative feed-back system, which acts to protect the body from the effects of complement activation by depressing the synthesis has been proposed (11, 17) but represents at present only a theory

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FOLLOW-UP

What happened to the patients?

Readers of scientific medical publications often complain about the fact that the paper is produced very soon after the patient has been observed and treated. This means that we do not get an overview of the whole disease but often just a glimpse of one particular stage.

The Editors have decided to introduce a special section in the Acta Medica Scandinavica under the heading FOLLOW UP WHAT HAPPENED TO THE PATIENTS?

Our authors are invited to give further information about the development of interesting cases whose history has been published previously The first report in this series is presented below

ACUTE LEUKEMIA AND ACHRESTIC ANEMIA IN A BROTHER AND SISTER

Jorgen Bichel

From the Institute of Cancer Research, Radium Centr Århus Denmark

In 1940 the author published an article entitled Acute leukemia and achrestic anemia in *brother and sister (Acta med. scand 104 578* 1940) The sister died of acute myeloid leukemia. The brother who suffered from a refractory megaloblastic-macroblastic anemia has later developed an acute leukemia so that the cases correctly should be classified as familial *leukemias*

Sponsored by the Danish Cancer Society

BOOK REVIEW

Third Conference on Cooley s Anemia The New York Academy of Sciences, Vol 232, 380 pages $33 00 New York 1974

Thalassemia—in this volume called Cooley s anemia—is a disease with a characteristic geographic pattern of distribution As a matter of fact Cooley s anemia is a misleading title of a book treating both the heterozygous and the homozygous states of the many different thalassemias. Cooley s anemia is still sometimes used as an eponym for the much rarer homozygous condition

Until recently even highly specialized hematologists have had very little personal experience from these interesting diseases if they work in Northern Europe During the last decade the gigantic migrations of foreign labour have brought people of Mediterranean origin to our latitudes. The present number of this journal contains descriptions Norwegian patients who may be examples of novo mutations leading to deficient synthesis either the α or the β chains in the globin molecule also in our part of the world

As a biological problem thalassemia has long been of great interest both to protein chemistry molecular medicine and genetics as well as to clinical hematology and geographical pathology This volume is the third in a series of conferences on the disease. It contains valuable information and stimulating discussion by leading specialists within the field and gives excellent illustrations of the fact that clinical medicine on the molecular level is a reality for millions of people

Already in the opening remarks the Chairman discusses the topical problem genetic engineering when he asks the question how to bypass the genetic defect. Different specialists on protein biosynthesis discuss the possible mechanisms for defective synthesis of certain polypeptide chains in this group of diseases It seems as if the function of messenger RNA were disturbed. Many of these papers seem to be far away from routine medicine even if they discuss fundamental problems in the etiology of common disease. There is however also discussion of such clinical problems as the dangers (iron overload) or advantages of massive transfusions the nature of urinary pigments in these diseases Interaction between different thalassemic defects and other genetically determined hemoglobinopathies or enzyme dificiencies and the question of intrauterine diagnosis

The chapter by Quattrin and Ventruto tells the story of a disease studied intensely in Naples and Southern Italy ever since it was first found in an Italian patient some 15 years ago This so-called Lepore hemoglobin is an experiment of nature that has taught as much regarding the interaction between different genes that determine the synthesis of globin polypeptides. The almost innumerable hemoglobinopathies are experiments by nature discovered at the bedside that cannot be repeated in the laboratory

The volume may be recommended to clinicians with an interest in fundamental medicine and—of course—to the biologist working in the basic sciences

Jan G Waldenstrom

Acta med scand Vol 197 pp 1 7–140 1975

MYOCARDIAL BIOPSY IN A CASE OF CARDIOMYOPATHY AND PARTIAL α-1 ANTITRYPSIN DEFICIENCY WITH LIVER ENGAGEMENT

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Abstract. A case of partial α-1 antitrypsin deficiency with liver engagement and biopsy-verified congestive cardiomyopathy is reported. The possibility of connection between these two diseases is discussed in view of recent literature

Since the genetic deficiency of α-1 antitrypsin and its association with emphysema was described by Laurell and Eriksson (12) increasing interest has been directed towards the significance of deficiency of this glycoprotein (11). Present in normal concentration in human serum it is a powerful inhibitor of a variety of proteolytic enzymes. Deficiency states may be homo- or heterozygous with a serum concentration of α-1 antitrypsin within the ranges of 5–85% of normal The incidence of homo- or heterozygous deficiency is estimated at approximately 1/30 in a normal population (7 8) The different deficiency states are classified according to the immunological phenotype in the Pi (protease inhibitor) system (8) where the homozygous deficiency is designated for instance PiZZ and the heterozygous PiMZ

Whereas the first reports described panlobular emphysema associated with homozygous α-1 antitrypsin deficiency it has later been shown that persons with partial deficiency are also prone to develop lung disease as well as cirrhosis (4 5). One case with α-1 antitrypsin deficiency in association with emphysema, angitis and glomerulonephritis has been described (16)

Recently we had the opportunity to investigate a patient with heterozygous α-1 antitrypsin deficiency with liver engagement and a congestive cardiomyopathy which gave rise to the question whether there might be a connection between these two diseases and the protease inhibitor deficiency

CASE REPORT

A man, born in 1924 who apart from cholecystectomy in 1962 has been in good health and working school porter In spring 1972 increasing fatigue dyspnea and ankle edema. The patient was considered to have developed heart failure and digitalis and diuretics were instituted with good but temporary effect Five months later the symptoms returned despite continuous treatment. The patient now seemed to be more seriously ill and was admitted to our department for cardiological examination Upon admission in Jan 1973 he showed signs of severe right heart failure with congested neck veins in the sitting position gross liver enlargement and ankle edema He complained of fatigue dyspnea as well as transient dizziness

Physical examination gave no evidence of valvular heart disease. BP 135/95 chest X-ray which some months earlier had shown a moderate but uncharacteristic heart enlargement was now normal On several occasions the ECG showed sinus rhythm with slight ST T changes corresponding to the left ventricle These changes could, however be attributed to the digitalis treatment. A bicycle ergometer test, starting with 4 min at 300 kpm/min, was discontinued at 600 kpm for 2 min because of dyspnea During and after the test certain accentuation of the ST T changes was noted Spirometry was performed with a Bernstein spirometer and normal values according to Berglund et al. (2) were applied. The functional residual capacity was measured by nitrogen washout method (3) The vital capacity and total lung capacity corresponded to 65% of predicted values and FEV 1.0% of vital capacity was 74 Consequently the patient had pronounced restrictive lung disease

Routine laboratory findings were normal except for elevated unconjugated bilirubin and spontaneously lowered prothrombin index to about 30% of normal. The hepatomegaly was further investigated by i infusion of Tc 99 m-sulphur colloid showing moderately enlarged liver and spleen. Liver biopsy showed histo-

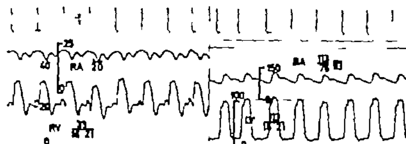


Fig. 1 ECG and pressure tracings from right and left heart catheterization in Jan 1973 *RA* = right atrium, *RV* = right ventricle, *LV* = left ventricle *BA* = brachial artery (mmHg). Upper tracing: ECG lead V_4. Paper speed 25 mm/sec.

chemical changes, consistent with α-1 antitrypsin deficiency with the typical rounded globules within the hepatocyte (4 18) which were resistant to treatment with diastase A routine lectrophoresis was normal with an α-1 antitrypsin concentration of 80 mg/100 ml A double crossed immunoelectrophoresis according to Laurell (11) was done, revealing that the patient had a partial α-1 antitrypsin deficiency of Pi type MZ.

Right and left heart catheterization was performed. The patient had pathologically elevated end-diastolic pressures in the right as well as the left ventricle and grossly elevated pressure in the right atrium (Fig. 1).

Pertinent values from this investigation and from another catheterization made 10 months later are given in Table I Angiocardiography showed normal pulsations in all heart chambers, normal valves and no signs of constrictive pericarditis. The pulmonary artery showed normal anatomy without signs of basal hypo- Semi-selective coronary arteriography revealed normal arteries and normal aortic valves. In connection with the catheterizations, endomyocardial biopsies according to Konno and Sakakibara (10) were done. On two occasions the sections from the right ventricle showed a rather widespread fibrosis and signs of hypertrophy (Fig. 2).

Large amounts of PAS-positive material occurred in and between the myofibrils This material wa glycogen since it disappeared upon treatment with diastase In some diastase-treated sections, however it was possible to recognize some small PAS-positive granules (Fig. 3). The appearance of these granules was not like those from this patient s liver and they were considered to be lysosomes. To exclude abnormalities in glycogen metabolism, biopsy from skeletal muscle was analysed and no such abnormality could be demonstrated.

The investigation thus ended in a case showing heart failure histochemical changes in the liver consistent with antitrypsin deficiency and a restrictive lung disease About one year later the spirometry was completed with a full pulmonary investigation including regional lung function measured by [133]Xenon radiospirometry (17). The values for perfusion per lung volume showed normal perfusion gradient between apices and bases in the sitting position. The typical picture of emphysema in association with α-1 antitrypsin deficiency implies an altered distribution of pulmonary perfusion with loss of this gradient. This method is considered the most sensitive for detection of early involvement of the pulmonary vascular bed (13)

The patient ha been treated orally with digitalis, diuretics and spironolactone since the first investigation. In addition he has received 20 mg furosemide and 250 mg theophylline t. twice a week.

Table I Values from the two catheterizations

RA = right atrium, RV = right ventricle PA = pulmonary artery PCV = pulmonary artery wedge LV = left ventricle BA = brachial artery (pressures in mmHg) HR = heart rate (beats/min) CO = cardiac output (l/min) SV = stroke volume (ml/beat)

	Jan. 1973	Oct. 1973
RA	20	13
RV	34	28
	16–21	10–16
PA	28 22	24 19
	20	15
PCV	19	19
LV	113	89
	16–21	11–15
BA	115 92	89 72
	78	61
HR	84	86
CO	3.86	4.59
SV	46	53

DISCUSSION

The connection between α-1 antitrypsin deficiency and emphysema has been known for more than a decade (6); cirrhosis of the liver has also been described in this protein abnormality (19), and as mentioned a single case with angitis and glomerulonephritis (16). Recently Campra et al.



Fig 2 Biopsy from the right ventricle showing widespread interstitial fibrosis and signs of hypertrophy of muscle fibres. Hematoxylin-eosin, ×116.



Fig 3 Biopsy from the right ventricle diastase-treated PAS staining. Arrows indicate PAS-positive granules, 291

(4) reported a case of cirrhosis associated with partial α-1 antitrypsin deficiency As far as we know antitrypsin deficiency has up to now not been reported in connection with primary myocardial disease The case presented here fulfils the criteria of a congestive cardiomyopathy (9) the etiology of which according to the definition is unknown

The pathogenic mechanism of the development of lung and liver disease associated with antitrypsin deficiency is not clearly understood Two separate theories have been put forward According to Eriksson (6 7) the antienzyme deficiency renders the tissue more vulnerable to damage by the lack of defence against liberated proteases. Lieberman et al (15) consider the position of intracellular antitrypsin to be of importance in developing the liver damage Lieberman (14) has also demonstrated the presence of degraded antitrypsin in the sera of patients with homo- or heterozygous antitrypsin deficiency This degraded antitrypsin has abnormal antigenicity which may give rise to antigen–antibody reactions causing tissue injury Whatever the mechanism may be several authors have stressed the importance of the damage of elastic tissue. As the hemodynamic investigations in this case have given values corresponding to loss of elasticity in the left as well as the right ventricle, it might be postulated that the supportive tissue of the heart has been damaged through lack of the inhibitory protein It seems improbable that α-1 antitrypsin deficiency alone can give rise to tissue damage since even individuals with homozygous deficiency have been protected from developing either the liver or the lung disease (18). It is certainly more likely that other unfavourable agents in inhibitor deficiency can initiate a chain of events leading to decreased function of an organ which is dependent upon its connective tissue This theory can be attributed to the heart as well as the lung provided that the myocardium has been exposed to for instance a myocarditis increased work load as in hypertension or toxic influence such as alcohol. These factors could ha e been present without clinical manifestations at the initial stage but nevertheless may have caused ir reversible changes in the myocardium. As the patient did not show any signs of emphysema of the lung his heart failure cannot be secondary to this disease

It is difficult to draw significant conclusions from a single case The combination of partial α-1 antitrypsin deficiency with pathognomonic changes in the liver and widespread fibrosis demonstrated in repeated biopsies from a severely damaged myocardium in this case of congestive cardiomyopathy gives rise to an hypothesis concerning its etiology α-1 antitrypsin has well documented protective roles in inhibiting the action of certain enzymes such as trypsin collagenase elastase and leucocyte proteases Se ere or intermediate deficiency of α-1 antitrypsi may render the myocardium more vulnerabl to noxious factors

which in the presence of normal antitrypsin concentration would not lead to any clinical consequences

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ERYTHRODERMIA SÉZARY WITH IMMUNOLOGICAL DEFICIENCY AND ANTIBODIES AGAINST HUMAN ALBUMIN

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Abstract A case of erythrodermia Sézary in which lymphocyte stimulation tests and quantitation of T and B lymphocytes were performed, is reported. The patient developed during 10 years of evolution an immunological deficiency with low IgA, IgG and IgM values. Passive hemagglutination techniques showed an antibody titer of 1 8000 against human serum albumin

Sézary and Bouvrain (17) described in 1938 a syndrome characterized by erythrodermia, pruritus leonine facies. They found in the peripheral blood atypical mononuclear cells which they believed belonged to the hystiocytes. Baccareda (1) described in 1939 a somewhat similar entity but claimed a "lymphomonocytic origin of the circulating cells. Following the original description a number of cases or series have been published all over the world and various interesting points have been reported We have had the opportunity to follow a patient who presented with the classical clinical and cytological features of the Sézary syndrome and besides slowly developed an immunological deficiency

METHODS

Routine hematological techniques have been used for the examination of the blood and bone marrow Duplicat samples were sent for cross-examination to the Centre d'Hématologie de Bordeau France (Prof J Moulinier). Quantitative immunoelectrophoresis determinations were performed at Malmo General Hospital (Prof C B Laurell)

In itro function tests and quantitation of T and B lymphocytes ere performed For these test heparinized blood was diluted 8 times ith Eagle medium containing 10% calf serum Streptomycin and penicillin were added Portions of the diluted blood (0 2 ml) were cultured in the wheels of Falcon Microtiter II plates Optimal amounts of mitogen or antigens ere added After 5 days incubation at 37°C 2 µC H-thymidine (5 Ci/mmol) from the Radiochemical Centre Amersham England was added Eighteen hours after the addition of H thymidine the cells w re washed on millipore filters. The filters were then dried and di solved in Soluen (Packard Instrument Co) and the radioactivity was determined in a Packard Ser 3000 Liquid Scintillation Spectrophotometer using Permablend® (Packard) in toluene. The quantitation of T and B cells was performed according to the rosette formation test as described by Jondal et al. (12) Demonstration of surface immunoglobulins was performed by indirect immunofluorescence on cells in suspension by the sequential addition of rabbit antihuman FAB (Behringwerke Marburg, Germany) and antirabbit immunoglobulin labeled with FITC (SBL, Stockholm, Sweden). The mitogen used were phytohemagglutinin (PHA) (Difco Lab Detroit), concanavalin A (Miles-Yeda Ltd Israel) and pokeweed mitogen (Grand Islands Biol Co., N.Y). Antigens used were PPD (Statens Serum Inst., Copenhagen) Monilia albicans (Hollister Stier Lab Spokane Wisc.), Varidase® (Lederle Lab) and Trychophyton Extr (N V Hall Allberg Lab, Harlem) Passive agglutination against bovine serum albumin (BSA) (lyophilized Hopkin & Williams) and human serum albumin (HSA) (lyophilized Kabi Sweden) was performed according to Boyden (7). Sheep red blood cells were coated with BSA and HSA respectively

CASE REPORT

The patient, 63-year-old woman, was first hospitalized in 1964 for "fever and chills The only clinical finding wa murmur over art. temporalis N palpable lymph nodes were found. The laboratory findings were ESR 71 mm/? h WBC 35 000/mm³ and normal differential count Bone marrow puncture showed no abnormality Serum electrophoresis showed "high α- but no high γ-globulins Mantoux was negative A biopsy of the art temporalis failed to disclose arteritis Urine culture was positive and streptomycin was tried but had to be stopped as the patient developed urticaria

She was then w ll until June 1973 (ith the exception of dermatophytosis of the feet and cataract) when she was rehospitalized with fever chills, pruritus and dyspnea. Clinical examination revealed erythema, most obvious on the dorsal side of the feet over the chest and on the face There



Fig 1 Electrophoretic migration pattern on paper



Fig 2 "Atypical lymphocyte" (Sézary cell) in the peripheral blood of the patient Giemsa, ×1000

was no local edema the local temperature was not increased and a fine furfuraceous desquamation could be seen over the same areas which were pruriginous. No palpable lymph nodes, liver or spleen were found. The laboratory findings were. Hb 11.9 g/100 ml WBC 24000/mm³ differential count showed 3% eosinophils and 7.5% atypical lymphocytes thrombocytes 510000/mm³ ESR 61 mm/1 h serum iron 114 μg/ml, TIBC 309 μg/ml, B_{12} 540 pg/ml, folic acid 2.3 ng/ml. The bone marrow showed normal erythropoiesis with normal sideroblasts but large iron deposits. The white granular series showed a moderate shift to the left and some more eosinophils. Some of the megacaryocytes were larger than normal. The conclusion of the laboratory was "reactive bone marrow with reservation concerning the giant megacaryocytes as an argument in favour of a myeloproliferative syndrome". Rheumatoid factors were negative.

The immunoelectrophoresis showed on several occasions low values for all the immunoglobulins. IgG 0.3–0.4 g/100 ml, IgA 0.02–0.05 g/100 ml IgM 0.03–0.05 g/100 ml, haptoglobin 225–360 mg/100 ml.

Skin biopsy presented hyperkeratosis, parakeratosis, light acantosis. Dermic infiltration with a moderate number of rounded cells. The conclusion of the pathologist was, "Chronic eczema? No certain evidence of mycosis fungoides".

Reexamination of the peripheral blood smear by one of us then revealed that the "atypical lymphocytes" mentioned by the laboratory were in fact similar to the Sézary cells which we have seen in a previous case (11).

Blood samples showed the presence of 30% of lymphocytes with T cell characteristics and 8% with B cell characteristics. A normal stimulation by PHA and concanavalin A was found. Immunofluorescence techniques showed that 7.5% of the cells had immunoglobulins on their membrane surface. The patient's serum was also titrated against HSA-coated sheep RBC in passive agglutination giving a positive titer of 1:8000.

Several attempts were made to treat the urinary tract infection but on all occasions and with all antibiotics used she developed urticaria. An asthma-like accident was also noted while on nitrofurantoin. Attempted treatment of the Sézary syndrome was made as others did too with Leukeran® 6 mg/day and prednisolone 30 mg/day. The clinical impression was favourable especially the effect on the pruritus, which disappeared, but the laboratory findings showed no changes and the treatment was stopped. She then began gradually to decline and developed signs of congestive heart failure although adequate amounts of digitalis and diuretics were provided. A thrombosis in the left leg was followed by an abrupt fall in the thrombocytes and she finally died.

The post mortem diagnosis was: Congestive heart failure endocarditis of aortic valve (old and new lesions). Several small emboli in the lungs. Thrombosis in the left vena iliaca. Cystitis. Cholelythiasis. The lymph nodes were not enlarged, on the contrary rather "hypoplastic". The spleen was described as "septic".

SOME CLINICO-PATHOLOGICAL ASPECTS OF THE SÉZARY SYNDROME

In the earlier reports there was a certain confusion about the clinical aspects of the disease, and one may feel that they included, under the same heading true cases of Sézary malignant diseases from the lymphoma group and other dermatological diseases. As new cases were reported a clearer picture emerged and by now the clinical signs and symptoms which are consistent with the diagnosis can be divided into: Constant ones: Erythroderma with exfoliation. Pruritus. Infiltration of the skin with "rounded cells". Presence of atypical lymphocytes in the blood. Normal bone marrow an important negative sign. *Inconstant ones*: Lymphadenopathy. Edema of the face ("leonine facies"). Hyperpigmentation (appears only by vitropression). Alopecia. Dystrophic nails (12). Hyperkeratosis of palms and soles. Hepatomegaly (12).

Biopsy of the skin

In nearly all the previously described cases there was great variation in the pathologist's diagnosis. Most frequently the skin was labelled as "chronic dermatitis" or "not

diagnostic: infiltration suggesting lymphoma" mycosis fungoides on one arm, reticulosarcoma on the other" etc. The conclusion is that there is no specific lesion of the skin in the Sézary syndrome.

The Sézary cell

The atypical circulating cell is described as mononuclear cell, with a large convoluted nucleu containing dense clumped chromatin and a scanty rim of cytoplasm. N nucleoli or mitoses have been reported. Lutzner et al. (15) have described two types of cells, large and small. In another study Lutzner and Jordan (16) have shown by electron microscopy that the nucleus appears cerebriform, ith overlapping folds and clefts, containing serpentine chromatin, often interconnected by narrow bridges. The cytoplasm contains mitochondria, ribosomes, polysomes and small amounts of endoplasmic reticulum. Histochemically peroxidase, alkaline phosphatase and non-specific cholinesterase were noted as "unremarkable" (15) which is important for the differential diagnosi against normal or leukemic monocytes which present strong positive peroxidase and non-specific cholinesterase reactions. PAS-positive reactions in the cytoplasmic granules ha been described but are not specific to the Sézary cell. Cytogenetic studies (15) have shown that the "large type" is hypo- or hypertetraploid and the small type hyperdiploid or pseudodiploid. Another study (8) showed in one case three cell populations, one normal (10%), one containing 70–76 chromosomes and distinct marker (70%) and third with 86–100 chromosomes and different marker (20%).

All over the world there is now considerable interest in studies of T and B lymphocytes. Recently Brouet et al. (4) using classical techniques but also an anti-T lymphocyte serum, were able to confirm in six cases that the Sézary cell bears T cell characteristics. U responsiveness of Sézary cells to PHA has also been reported (4).

Course and prognosis

The life expectancy in the Sézary syndrome i commonly five years from the time of diagnosis. There are ery few reported post-mortem examinations (2, 3). Those available have not disclosed any specific modification in any organ, including lymph nodes or spleen. No metastatic lesions or leukemia have ever been found. Some imprints of lymph nodes are processed and revealed the presence of free Sézary cells. The alleged cause of death is usually congestive heart failur or "infections.

COMMENTS

The purpose of this paper is to describe the case of an 83-year-old woman with the Sézary syndrome. The diagnosis was based on the presence of erythroderma, pruritus, presence of Sézary cells in the peripheral blood and in the skin together with a normal bone marrow.

We found that 30% of the patient s lymphocytes had T cell characteristics. Other recent studies (14) using an anti-T cell serum demonstrated that the Sézary cells seem in fact to be T cells. They also co-operate with B cells in the production of various immunoglobulins. There is actually no proof that the Sézary cells behave abnormally but it may be so. Cottier et al. (7) recently proposed a new classification of lymph node pathology based on identification of T and B cells. This is a new approach and s h a study of Sézary would be interesting. The pecial features of our case worthy of comment were the hypogammaglobulinemia and the presence of antibodies against HSA.

Electrophoretic studies of serum from patients with Sézary syndrome have seldom been mentioned in the literature. Brody et al. (3) described a case as "unremarkable" and another as having reduced serum albumin". Crossen et al. (8) described one case as normal" while Brouet et al. (4) found high IgM in one of six patients. We therefore consider the immunological deficiency of our patient remarkable. This was not present in 1964 but was fully developed in 1973. The choice had to be made between a primary deficiency of late onset (common type or " ommon variable) which is usually related to autoimmune disorders or a secondary deficiency. The latter is often found in patients uffering from lymphatic or other malignancies. In fact some authors (13) seem to consider the Sezary syndrome a rare chronic lymphatic leukemia. We think that this is rather speculati e. As our patient failed to show any signs of leukemia or malignancy at post mortem we consider that immunological deficiency was of primary type in this case. Normally the B lymphocytes are those which synthetize and secrete Ig. Some think that the T cells timulate (directly) or co-operate with the B cells to this end. As Choy et al. (5) pointed out the decrease in Ig synthesis or failure of Ig secretion may be explained by a block in differentiation or maturation of B cells and subsequent arrest at a presecretory stage. We can but peculate that the Sézary cells have acted in this way.

The high titer against HSA is also remarkable and indicates break of tolerance. Animal experiments suggest that tolerance termination or circumvention can be caused by the administration of heterologou protein or homologous protein coupled to hapten. Antibody to thyroglobulin and subsequent thyroiditis has been induced n rabbits by immunization with hapt n-conjugated homologous thyroglobulin (18). In our case the drugs to which the patient reacted clinically may have functioned as a

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hapten, combining with the patient's albumin and resulting in a carrier conjugate. Alternatively tolerance termination can be induced by BSA in the food. Antibodies against BSA have been reported in rabbits after oral administration (10) but they are not a usual finding in man. Although BSA and HSA cross-react human antibodies against BSA are normally directed only against antigenic determinants not present on HSA. (Unfortunately absorption studies could not be performed as too little serum was left.)

Whether the Sézary cell itself is involved in this abnormal reaction against self-protein is left as an open question.

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EDITORIAL

SPLENECTOMY IN CHRONIC MYELOCYTIC LEUKAEMIA

Ever since the turn of the century the question of the advisability of splenectomy in cases of chronic myelocytic leukemia (CML) has been discussed with waxing and waning interest. Times when such a procedure has been advocated on more or less well founded arguments have alternated with periods when splenectomy has been condemned as contraindicated and potentially dangerous

Reports have been published on occasional cases successfully subjected to this operation (1 2, 5 8 9) and larger reviews have also tried to elucidate the accumulated experience in the literature (1 7 12)

On the whole, such reviews have resulted in a rather pessimistic view as a large percentage of the operated patients seem to have gained very little benefit from the operation. Admittedly these relatively early reports suffer from the fact that the operation at that time was usually undertaken as a last resort when the patient after conventional medical treatment had failed was on a downhill course with severe anaemia, sometimes also leukopenia and bleeding tendency due to advanced thrombocytopenia (1)

As the general opinion seems to have changed considerably during the last few years and the operation is now performed under somewhat different indications in an increasing number of CML cases and even as an almost initial procedure it may be in place to give an account of the recent results emerging from the literature and from congress reports.

The situation at present is quite different from what it was only a few decades ago More effecti e antileukaemic drugs have made it possible to achieve a rapid initial remission in most cases of CML there is a much greater possibility of coping successfully with infections that were often fatal before, the blood center service is much more elaborate today not only as regards conventional blood transfusions but also in the selective transfer of concentrates of e g platelets. All these means have helped to reduce the immediate postoperative mortality to acceptable levels and have led to the concept of elective surgery in suitable cases and when strict indications for the operation are otherwise present.

Thus the reluctance to perform splenectomy in CML felt by experienced investigators earlier (7 8 12) has decreased considerably during the last ten years or so Many people have appreciated the philosophy of Crosby put forward in discussions over splenectomy in haematology in general "Splenectomy should be done when the patient does not need it. If one waits until the patient needs the operation he may not be able to tolerate it" (3).

The earlier indications included massive splenomegaly with splenic infarction and/or unbearable discomfort from the enlarged spleen and the development of hypersplenism with pronounced anaemia and low platelet counts. In the splenectomized CML cases who survived the operation a rapid return of the red cell count to normal and an increase of the thrombocytes were regularly seen In addition the patient s susceptibility to cytostatic drugs was also demonstrated to increase after the operation Lower dosage of such drugs was often sufficient to keep the patient in a good condition and with a leukocyte count within the normal range in exceptional cases all cytostatic treatment could be withdrawn for a considerable time after the operation (4).

To these indications can be added others that are even more important. The enormously enlarged spleen harbours a very great proportion perhaps trillions, of the pathologic cells in the body The operation removes this major fraction of cells in one step and if the patient has been treated initially with an appropriate course of antileukaemic drugs resulting i reduction of the spleen and the best possi-

ble general condition, further management may be considerably facilitated and a substantial prolongation of a remission can be anticipated with or without small doses of cytostatic drugs (1 10)

Zubrod has also suggested early splenectomy as a means of removing initially as much of the pathologic cell population as possible· thereafter he advocates intensive antileukaemic drug treatment in the hope that eventually all pathologic cells will be eradicated

Furthermore based on supporting evidence (11), it has been claimed that in the spleen a great number of malignant clones of cells can be found during the transition of a case of CML into the final acute leukaemia phase so that the early removal of a potentially dangerous cell population might postpone the acute blastic crisis. This has been explained as the metamorphosis of a conditioned neoplasm into an autonomous one (10) Moreover the spleen can constitute an important hiding-place for leukaemic cells protecting them from the action of cytostatic drugs

In Sweden an early splenectomy has been advocated for several years (1) as a means of better management of cases of CML and with the goal of facilitating the further control of the disease A limited number of splenectomized cases supports the advisability of such a procedure with reference to the better state of the patient and a robably prolonged survival

Spiers and co-workers in Britain (10) have re ntly reported their opinion regarding elective splenectomy early in the course of CML, based on a controlled pilot study of 17 cases which has already run for several years. They have formulated a number of reasons for undertaking the operation early in the course of the disease They feel that there is a good case for early splenectomy in CML as a prophylactic measure against almost inevitable problems which will arise later in the course and as a means of certainly increasing both the quality and the length of the patients life Galton and Spiers (6) had already stressed that splenectomy is an excellent way of drastically reducing the total mass of leukaemic cells which also makes it possible to expose the patients to a minimum quantity of antileukaemic drugs later on. These have been shown in some cases to affect adversely the further evolution of the disease (6) If it eventually turns out that the spleen is in fact a place for the development of new anaplastic clones of cells capable of initiating the transition of CML into acute leukaemia, additional advantage will be gained by an early operation.

Likewise Baikie who earlier worked with Spiers in London has performed early splenectomy in all cases of CML since 1965 in which no special contraindications were present. Although this was not a controlled trial he claims that his preliminary results suggest a significant prolongation of life after the operation and he also holds that this procedure not merely removes the major extramyeloid site of cell proliferation and cell accumulation, but also has a favourable influence on the natural history of the disease and especially the occurrence of metamorphosis or even acute transformation.

Now that an extensive controlled multicenter trial has been started under the auspices of the British Medical Research Council with the aim of evaluating the effects in cases of CML of early splenectomy on the length and quality of the patient s survival haematologists all over the world await the results with eager interest

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NEUTROPHIL GRANULOCYTE FUNCTION IN THE EARLY DIAGNOSIS OF ACUTE MYELOMONOCYTIC AND MYELOBLASTIC LEUKAEMIA

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Abstract The phagocytic and bactericidal activities of neutrophil granulocytes from 5 patients with early acute myelomonocytic or myeloblastic leukaemia and 5 controls have been examined. In each patient the bactericidal activity was lower than in any control and the neutrophil dysfunction was demonstrated before leukaemia could be diagnosed from clinical and haematological findings. During periods of remission, the bactericidal activity was normal. Results of neutrophil granulocyte function studies may be a significant aid in the early diagnosis of acute myelomonocytic and myeloblastic leukaemia

In many cases the diagnosis of acute myelomonocytic or myeloblastic leukaemia is easy The clinical picture calls for haematological examination which reveals the typical findings of anaemia thrombocytopenia and large numbers of blast cells in the peripheral blood and bone marrow However during the early stage of the disease, when clinical symptoms are less pronounced haematological data may only show minor alterations from normal values and the diagnosis may remain uncertain for months. In these cases we have found the results of neutrophil granulocyte function studies to be a significant diagnostic aid

One of us has developed a method which facilitates a precise in vitro evaluation of the phagocytic and bactericidal activities of neutrophil granulocytes (18, 19) Using this method a significant reduction in neutrophil granulocyte function has been demonstrated in each of 5 patients with early acute myelomonocytic or myeloblastic leukaemia when the diagnoses based on clinical and haematological findings were inconclusive

MATERIAL AND METHODS

Patients and controls

Leucocytes were obtained from 3 male and 2 female patients, aged 18–54 years, during early acute myelomonocytic (3 patients) or myeloblastic leukaemia. The diagnoses based on clinical and haematological findings could not be substantiated until 3– weeks (mean 6) later Three patients had experienced fever and malaise for 1–3 weeks and two patients sore throat and lymphadenitis for 1– weeks. In addition to fever tonsillitis and lymphadenitis, clinical examination revealed signs of anaemia in 2 patients Haematological data, including results of 3 peripheral blood smear and buffy coat examinations and 3 sternal bone marrow aspirate examinations from each patient, did not substantiate the diagnoses (Table I). Auer bodies were not observed Results of alkaline phosphatase reaction (6, 9) in neutrophils and PAS staining (6) in blast cells were within normal limits Serum muramidase estimation (17 22) were performed in 4 patients and the results were normal Normal peroxidase reaction (10) was demonstrated in 30–60% of peripheral neutrophil granulocytes, the remaining peripheral myeloid cells showing little or no reaction Segmented neutrophils and neutrophil precursors in bone marrow smears also showed diminished peroxidase reaction

Leucocytes obtained from staff members of the Department were used as controls for comparison of phagocytic and bactericidal activities, and for each test with patient leucocytes control test was performed

Leucocytes

Leucocytes obtained by Isopaque®/dextran sedimentation of heparinized venous blood (10 U heparin/ml blood) were washed twice in heparinized saline (1 U heparin/ml saline) by centrifugation at 500 *g* for 5 min (18). A differential count was performed after the final centrifugation and the cells were resuspended in Hanks' balanced salt solution containing 0.1% gelatin to make concentrations of 10^6 neutrophils (band-form and segmented) per ml.

Bacteria

Staphylococcus aureus Oxford (Heatley strain, obtained from the National Collection of Type Cultures, Colindale, London 1958) was used as the test organism (18). The bacteria were cultured overnight in Penassay broth (Difco) washed twice in 0.45% saline and suspended in Hanks' balanced salt solution to an optical density of 0.6 at 620 nm in Beckman spectrophotometer This suspension was diluted in Hanks' balanced salt solution containing 0.1% gelatin to concentration of 13 10^7 17×10^6 colony forming units per ml.

Table I *Laboratory data from 5 patients with early acute myelomonocytic or myeloblastic leukaemia (mean values within parentheses)*

Peripheral blood

Hb concentration (g/100 ml)	7.3–14 (11.3)
Erythrocyte count (mill./mm³)	2 4–4 9 (3 9)
WBC/mm³	3 200–13 500 (7 300)
Differential count (%)	
Blast cells	0
Myelocytes	0–13 (7)
Granulocytes	42–69 (58)
Lymphocytes and monocytes	23–40 (35)
Thrombocyte count/mm³	128 000–584 000 (308 000)

Sternal bone marrow aspirates

Normocellular*	6/10
Slightly hypercellular*	4/10
Blast cells (%)	1–8 (4)
Promyelocytes (%)	3–10 (6)
Myelocytes and metamyelocytes (%)	31 70 (52)
Myeloid-erythroid ratio	4–19 1 (8 4 1)
Megakaryocytes present	10/10

No of aspirates/total no of aspirates

Serum

Volumes of 1 ml pooled fresh normal serum from 7 adults were stored at −30°C Immediately before each experiment 1 ml freshly thawed serum was mixed with 3 ml Hanks balanced salt solution containing 0 1% gelatin

Leucocyte-bacteria suspension

Leucocyte suspension, 0 5 ml bacteria suspension 0.1 ml and diluted serum, 0 4 ml were added to 12×75 mm posable plastic tubes This provided about 3 bacteria per trophil granulocyte and a final concentration of 10% The tubes were incubated at 37°C with an end-r-end rotation to promote contact between bacteria and leucocytes Samples were removed at prescribed intervals for determinations of the total number of iable bacteria and the number of iable intracellular bacteria. The bactericidal capacity of the granulocytes is proportional to the total number of bacteria killed and inversely proportional to the total number of viable bacteria or the number of viable intracellular bacteria (19) The number of bacteria phagocytized equals the number of viable intracellular bacteria plus the number of bacteria killed (19).

The total number of viable bacteria wa determined after osmotic disruption of the leucocytes by adding 0 01 ml of the leucocyte-bacteria suspension to 1 ml distilled water Quantitation of viable bacteria was made from appropriate dilutions of this suspension using a standard pour-plate technique.

The number of viable intracellular bacteria was determined by the technique of Solberg (18). 0 01 ml of the leucocyte-bacteria suspension and 1 ml Hanks balanced salt solution containing 0 1% gelatin, 500 μg streptomycin, 500 U penicillin G and 2 mg phenylbutazone were incubated at 37°C for 15 min and centrifuged for 10 min at 500 g The cellular pellet was washed twice in Hanks balanced salt solution and resuspended in 1 ml distilled water for osmotic disruption of the leucocytes. Viable bacteria were counted by the pour-plate technique. The results for each subject are recorded as the mean of 3 experiments one on each of 3 consecutive days

RESULTS

In the test with neutrophils from the controls a marked reduction in viable bacteria was observed, especially during the early phase of incubation and only 0.8–2.0% (mean 1.3%) of the bacteria remained viable after 120 min (Fig. 1). In contrast 4.8–11 9% (mean 7 3%) of bacteria in the tests with patients neutrophils remained viable after incubation for 120 min and large numbers of viable bacteria were located intracellularly (Fig. 2) indicating normal phagocytosis but reduced intracellular killing of bacteria When the patients condition deteriorated and more immature cells appeared in the bone marrow and peripheral blood the difference in granulocyte function between patients and normal controls became even more pronounced and finally the number of morphologically normal granulocytes was reduced to such an extent that the test could not be carried out

Following antileukaemic therapy 1 patient had two complete remissions and 2 patients one remission each. During periods of complete remission results of granulocyte function tests were normal. Two patients could be followed for several months by frequent granulocyte function studies and haematological examinations. Reduced granulocyte function was demonstrated as soon as immature cells appeared in the peripheral blood and, in one instance several days before change in normal blood picture Clinical symptoms were not present at the time of these early relapses

DISCUSSION

Several syndromes characterized by chronic bacterial or fungal infections have been related to defects in the microbicidal capacity of neutrophil granulocytes (3 5 8 12, 14 15 21). The first syndrome to be described was chronic granulomatous disease which is characterized by the occurrence in male children of dermatitis lymphadenitis hepatosplenomegaly chronic suppurative infections and granuloma formation (4) Persistent defects in neutrophil bactericidal capacity with clinical mani-

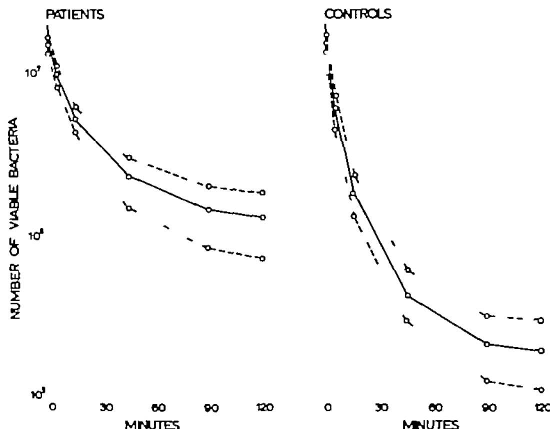


Fig. 1. Total number of viable bacteria during incubation with neutrophil granulocytes from 5 patients with early acute myelomonocytic or myeloblastic leukaemia and from 5 controls. — =mean, - - =range.

festations of varying severity and with different genetic patterns have been included later in this syndrome (5, 8, 14, 21), indicating that the term chronic granulomatous disease covers several different types of neutrophil dysfunction. Myeloperoxidase (11, 12) and glucose-6-phosphate dehydrogenase (3, 14) deficiencies may also result in persistent neutrophil dysfunction and chronic infections. In addition, temporary dysfunction has been demonstrated, especially in patients with severe bacterial infections (13, 20), extensive burns (1, 2) and after acute trauma (1). Finally, as demonstrated in the present study, reduced granulocyte function may also be present during the early stage of acute myeloblastic and myelomonocytic leukaemia even before the diagnoses can be substantiated by clinical and haematological findings. Granulocyte function tests may, therefore, be of significant value in the early diagnosis of these malignancies.

The differential diagnosis between disorders of persistent neutrophil dysfunction and early acute leukaemia may sometimes be difficult. However, the former disorders are usually inherited (3, 5, 8, 12, 14, 15, 21), and in chronic granulomatous disease the insidious onset during early childhood, the typical clinical picture of dermatitis, lymphadenitis, hepatosplenomegaly and chronic suppurative infections despite normal hematological findings should not be misinterpreted as acute leukaemia even in its very early stage. Besides, neutrophil dysfunction is more severe in chronic granulomatous disease. In glucose-6-phosphate dehydrogenase deficiency, hemolysis is a typical finding, the myeloid series is normal and results of biochemical erythrocyte analyses will give the diagnosis (3, 14). In myeloperoxidase deficiency, staining methods containing for example benzidine will demonstrate *depletion* of cytoplasmic myeloperoxidase (12, 14). Besides, these patients seem particularly susceptible to infection with *Candida albicans* (12, 14). Finally, bacterial infection may sometimes be associated with transient neutrophil dysfunction (13, 20) and the

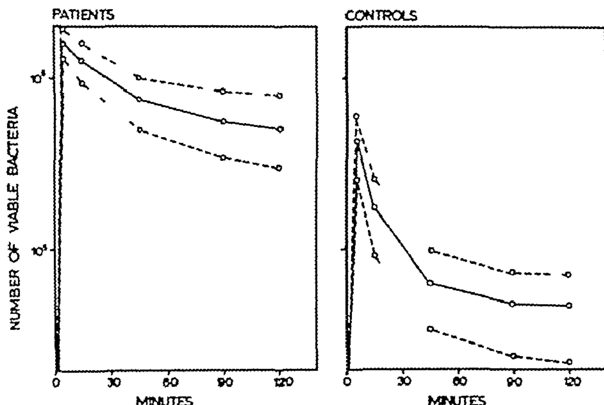


Fig 2 Number of viable intracellular bacteria during incubation with neutrophil granulocytes from 5 patients with early acute myelomonocytic or myeloblastic leukaemia and from 5 controls. Symbols as in Fig 1

blood picture may show leukaemoid reaction. In these cases results of neutrophil granulocyte function tests will soon improve and return to normal values during adequate antimicrobial therapy (20)

We do not know why neutrophil granulocyte function was reduced in our patients. The killing of phagocytized microorganisms by neutrophil granulocytes involves a complex series of biochemical events (5 11 12 16) and it is likely that several interlocking killing mechanisms exist within each cell (5 11 16) However as more information has been gathered during recent years correlating neutrophil bactericidal activity and neutrophil metabolic response to phagocytosis it has become apparent that hydrogen peroxide plays an important role in the intracellular killing of microorganisms (5 11 14 16). Between them myeloperoxidase a halide and hydrogen peroxide form a strong antimicrobial system (11 16) and in chronic granulomatous disease the bactericidal defect is characterized by reduced postphagocytic oxygen consumption and hexose-monophosphate-shunt activity resulting in reduced hydrogen peroxide production (5 11 14 16). The neutrophil dysfunction in glucose-6-phosphate dehydrogenase deficiency seems also to be due to deficient hydrogen peroxide production (3 14) and in myeloperoxidase deficiency to absence of the enzyme itself (11 12, 14). In diseases with temporary neutrophil dysfunction, intracellular enzyme defects have not been reported. However in previous studies of patients with bacterial infections (20) we found low numbers of peroxidase-reacting granules more often in granulocytes from patients with reduced neutrophil granulocyte function than in patients with normal function Leucocyte myeloperoxidase deficiency Candida infection and reduced rate of intracellular killing of bacteria by the granulocytes have also been described in a patient with myelomonocytic leukaemia (7).

Whether the reduction in peroxidase-reacting granules demonstrated in the granulocytes from our leukaemia patients is the ultimate cause of the neutrophil dysfunction in early leukaemia or only part of a faulty differentiation of enzyme synthesis remains to be determined.

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INTRAUTERINE DEATH AND CIRCULATING ANTICOAGULANT (ANTITHROMBOPLASTIN ')

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Abstract. A report is presented of young, otherwise apparently healthy woman who had had three pregnancies which for some unknown reason terminated in intrauterine death (macerated foetuses) During the third pregnancy a coagulation defect was diagnosed which was characterized by prolonged coagulation times and prolonged one-stage prothrombin time This defect disappeared after the end of the pregnancy but returned during the fourth pregnancy This time circulating anticoagulant was found, which inhibited the action of thromboplastin. The values found for the various coagulation factors were normal. The anticoagulant titre rose during the pregnancy from 1/2 to 1/10 Leucocyte agglutinating as well as lymphocytotoxic antibodies directed against the husband's cells were demonstrated in the patient during the pregnancy. In this case by passage of cell fragments and thromboplastic substances to the mother the foetus had probably induced the development of antibodies against the foetal tissues. The foetu may be regarded as an incompatible transplant. The fourth pregnancy was terminated by caesarean section in the 34th week. The child weighed 1 440 g and, after three exchanges of blood, did very well. The placenta was severely infarcted. It is postulated that the development of antithromboplastin during pregnancy may be a contributory cause of intrauterine death.

Circulating anticoagulants may develop spontaneously even in the absence of primary haemophilia. Such anticoagulants have been demonstrated in patients with various autoimmune diseases such as SLE, rheumatoid arthritis, drug allergy in women after childbirth and sometimes in apparently healthy middle-aged and elderly persons (2 4 6 9 18 21 26) The anticoagulants may be directed against factors V VIII IX XI XII, prothrombin and thromboplastin. The occurrence of circulating anticoagulants is characterized by prolonged coagulation times and, clinically often by a tendency to haemorrhagic diathesis

A description is given of a young woman who had had three pregnancies, each of which terminated in intrauterine death for which no explanation could be found During the third pregnancy examination revealed a coagulation defect characterized by prolonged coagulation times and prolonged one-stage prothrombin time. This defect disappeared after the pregnancy but recurred during a fourth pregnancy It was then found that she had a circulating anticoagulant inhibiting the action of thromboplastin Thanks to careful observation and timely induction of deliv ery by caesarean section the woman gave birth to a healthy child.

METHODS

Platelet and coagulation studies. Determinations of the platelet count, Duke and Ivy bleeding times, platelet adhesiveness according to Hellem whole blood method, coagulation time in glass and plastic tubes, recalcification time, partial thromboplastin time, one-stage prothrombin time (human brain thromboplastin), Stypven time factor VIII factor IX, factors XI and XII, prothrombin, factor VII and factor X (Owren P&P test), factor V fibrinogen, thrombin time and antithrombin III (biological and immunochemical method) were made by the procedures described earlier (5 12, 19 24, 26).

T st for circulating anticoagulant and normalization tests The inhibiting effect of the patient plasma on the recalcification time of normal plasma was determined. The highest plasma dilution capable of prolonging the recalcification time by 15 sec was taken as the anticoagulant titre of the patient s plasma. Normalization tests were carried out in the same way with the patient' plasma as test base (23).

Two-stage methods for assay of inhibitors against factor VIII and factor IX These tests were performed in the way described by Holmberg and Nilsson (14) and Nilsson et al. (20). *Inhibitory activity against factors XI and XII* was determined according to Åberg and Nilsson (36) *Anticoagulant activity in the presenc of thromboplastin*

Table 1 *Results of coagulation studies*

	Third pregnancy week 31 11/68	Non-pregnant 7/69	Fourth pregnancy			
			Week 22 12/71	Week 27 1/72	Week 34 3/72	3 d. after delivery
Coagulation time (min)						
Glass	25	13	22	32	28	27
Plastic	60	34	>60	>60	>60	60
Platelet number (per mm³)	120 000	290 000	176 000	240 000		
Platelet adhesiveness (%)						
HeRem	23	33	27			
Bleeding time (min)						
Duke		4	4			
Ivy			12	12		
Recalcification time (sec)	480	179	300	460	470	260
One-stage prothrombin time (sec)	21	16	20	22	20	18
Stypven time (sec)			33			
Partial thromboplastin time (sec)		90	180	287	320	218
P&P (%)	90	105	108	112		
Factor V (%)	95	74	86	92		
Factor VIII (%)	139	105	202	170		
Factor IX (%)			137	130		
Factor XI (%)			90			
Factor XII (%)			100			
Thrombin time (sec)	20	20	17			
Fibrinogen (g/100 ml)	0.49	0.36	0.55	0.51		
Anticoagulant titre		0	1 2	1 10	1 10	1 5
Antithrombin III (%)						
Biological method		100	120			
Immunochemical method		100	100			

The inhibiting effect of the patient s plasma on the coagulation time of normal plasma in the presence of various concentrations of human brain thromboplastin (prepared as described by Owren (25) and Astrup et al. (1)) was determined. Normalization tests were done in the same way with the patient s plasma as test base. The clotting system: 0 2 ml normal citrated plasma or patient s plasma+0.2 ml normal citrated plasma dil. 1/5 1/10 1/20 or 0.2 ml patient's plasma dil. 1/5 1/10 1/20+0.2 ml thromboplastin in decreasing concentrations (dil. 1/1 1/10, 1/100 1/1 000, 1/10 000) were mixed and incubated for 3 min in a water bath at 37°C, after which 0 2 ml 30 mM $CaCl_2$ solution was added and the clotting time was measured The same tests were performed with Russell viper venom (Stypven Burroughs Wellcome & Co London) instead of thromboplastin.

The thromboplastin generation test according to Biggs et al (3) was modified in that Thrombofax (General Diagnostics) was substituted for the platelet suspension. The intrinsic thromboplastin formed using normal adsorbed plasma (dil. 1/5) and normal serum (dil 1/10) was tested simultaneously on normal platelet-poor citrated plasma, patient s platelet-poor citrated plasma and a mixture of 4 parts normal and 1 part patient platelet-poor citrated plasma, respectively

Fibrinolytic studies The following determinations were made: fibrinolytic activity of plasma and resuspended euglobulin precipitate on fibrin plates, plasminogen (immunochemical method), inhibitors of plasminogen activation (urokinase inhibitors), α_2-macroglobulin and fibrin degradation products (FDP). The methods have been described previously (11 13 22).

Histocompatibility testing Lymphocytotoxic antibodies were determined according to Kissmeyer-Nielsen and Kjerby (16) and leucocyte agglutinating antibodies according to Kissmeyer-Nielsen (15)

CASE REPORT

A 25-year-old woman without heredity of haemorrhagic disease. In 1967 she had phlebographically verified thrombosis of the right leg during use of oral contraceptives of combined type.

In 1966 the patient was pregnant for the first time she was then 19 years old In the 31st week of pregnancy intrauterine death occurred and the patient was delivered of a macerated child 37 cm long. No explanation could be offered for the intrauterine death. Routine histological examination of the placentra revealed nothing remarkable.

In 1967 the patient was again pregnant she was then 20 years old Also that pregnancy terminated in intrauterine foetal death in the 22nd week. The patient was delivered of a severely macerated foetus 26 cm long No explanation could be offered for the intrauterine death. Routine histological examination of the placentra showed widespread necrosis and calcium deposits.

In 1969 the patient was pregnant for the third time she

Non-pregnant (mo. after delivery)				
1 4/72	5 8/72	9 12/72	23 2/74	Normal range
38	32	28	36	6–14
53	54	90	100	12–32
			240 000	150 000–300 000
				17–33
				1–5
				6–12
280	230	220	300	120–180
17	16	16	21	13–15
			50	19
		160	250	90–120
			90	80–120
			113	60–140
			105	60–140
			95	60–160
				60–160
				60–160
				18–20
			0.34	0 20–0.40
1 5	1 2	1 1	1 2	
				60–140

was then 22 years old In the 29th–30th week the patient complained of chest pain and thromboembolism was suspected. The diagnosis was not verified, but the patient was treated for short period of pregnancy with heparin and dicoumarol. The pains were afterwards localized to the region of the liver and the serum bilirubin rose to 1.5 mg/100 ml. An increase in diastase was noted as well as increased SGOT (310 U) and SGPT (500 U). Cholecystectomy revealed nothing remarkable. Intrauterine death occurred immediately after these episodes; the patient was then in the 34th week. She was delivered of slightly maccrated foetus 34 cm long. Routine histological examination of the placenta showed widespread necrosis and abundant fibrin deposits. During her third pregnancy the woman also had Rh immunization (anti-c), but with such a low titre that it was not considered to be of clinical significance. During that pregnancy the coagulation status of the patient had been investigated. A coagulation defect was found with prolonged coagulation times in both glas and plastic tubes and prolonged one-stage prothrombin time, while the values found for the various coagulation factors were normal.

At reexamination 3 months after delivery both the coagulation times and the one-stage prothrombin time were normal.

In 1971 the patient was pregnant for the fourth time she was then 24 years of age She had her last menstruation on July 19 1971. Pregnancy was at first apparently normal At examination in the 21st–22nd week the coagulation times were again prolonged and were found to be due to an anticoagulant.

The patient was examined for any underlying disease Examination for rheumatoid factors was negative Antinuclear factor (ANF) was demonstrated in titre of 1/512. No LE cells could be demonstrated with certainty Immunoelectrophoretic analysis revealed IgG 1 100 IgA 120 and IgM 90 mg/100 ml all within normal limits. It was found that the patient serum contained leucocyte agglutinating as well as lymphocytotoxic antibodies, which were directed also against the husband' cells. Leucocyte agglutinating antibodies were also demonstrated in amniotic fluid obtained by amniocentesis. Rh immunization against c was also demonstrated. Anti-c-titre rose from 1/32 to 1/512 (papain). The patient, who belonged to blood group O Rh+ proved to be genotype CDE/CDE. The husband belonged to blood group Rh− with genotype cde/cde.

In the further course of the pregnancy the patient showed no signs of toxicosis. She had no oedema or proteinuria and the BP was normal. The excretion of oestriol in the urine was, however always somewhat low suggesting placental insufficiency Biparietal measurements by means of ultrasonics showed also retarded growth. No treatment was given during pregnancy

In the light of the earlier obstetric history and the above mentioned laboratory findings it was decided to deliver the patient between the 34th and 35th week of pregnancy Caesarean section was done on March 17 The child, a girl with a birth weight of 1440 g, had an Apgar score of 9 points. The placenta showed abundant infarcts. The histological examination revealed signs of bleeding, necrosis and fibrin deposits.

The child was found to belong to blood group O Rh+ Coombs test was positive. Serum bilirubin was 7 1 mg/100 ml and was not allowed to exceed 10 mg/100 ml. The three blood exchanges performed were uncomplicated and the further development of the child was normal except for some feeding difficulties. The child left hospital on April 20, 1972, and her development has since been normal. The mother had no complaints during the puerperium

RESULTS

Coagulation studies

The results of the coagulation studies are summarized in Table I At first examination during the third pregnancy (in 31st week) the platelet count was somewhat low Platelet adhesiveness was normal. She had markedly prolonged coagulation times in glass and plastic tubes and prolonged recalcification and one-stage prothrombin times The values for P&P factor V factor VIII fibrinogen and thrombin time were normal. At that investigation no tests were made for circulating anticoagulants The patient had no signs of fibrinolysis and the values for the various components of the fibrinolytic system were normal (Table II).

Table II *Data on the fibrinolytic system*

	Third pregnancy	Non-pregnant	Fourth pregnancy			Non-pregnant (mo. after delivery)				Normal range
			Week 22	Week 27	Week 34					
	11/68	7/69	12/71	1/72	3/72	1	5	9	23	
Spontaneous fibrinolytic activity (lysed area in mm²)										
Plasma	0	0	0	0	0				0	0–30
Resusp euglob prec.	0	38	0	0	0				0	0–70
FDP (μg/ml)	0	0	0	0	35	10	0	0	6	0–5
Plasminogen (%)	131	91	95							60–140
Inhibitors of plasminogen activation (%)	67	1 1	114							60–140
α_2-macroglobulin (%)	134	112	93							80–120

At reinvestigation 8 months later when the patient was not pregnant, she had a normal coagulation time recalcification time and one-stage prothrombin time No anticoagulant activity could be demonstrated

During her fourth pregnancy the coagulation status was continually followed from the 22nd week of pregnancy In the 22nd week she again had a prolonged coagulation time recalcification time one-stage prothrombin time and also Stypven time e factor VIII IX XI XII V prothrombin (P&P) fibrinogen levels were normal for the stage of nancy She had a normal antithrombin III level nd normal thrombin time. The patient s plasma in dilution 1/2 prolonged the recalcification and one-stage prothrombin times of normal plasma. Addition of normal plasma failed to normalize the prolonged coagulation times These findings indicated an anticoagulant interfering with thromboplastin and/or the prothrombin activation The anticoagulant titre increased during the pregnancy to 1/10

The components of the fibrinolytic system were normal. FDP were not present until just before delivery

Three days after delivery the anticoagulant titre was 1/5 Nine months later the anticoagulant was still present, but the titre had decreased to 1/1 At investigation 2 years after the delivery the anticoagulant titre had increased to 1/2.

Properties of the anticoagulant

Addition of the patient s plasma to normal plasma increased the recalcification time and the partial thromboplastin time of normal plasma. The highest dilution at which the inhibitory effect of the patient s plasma at the end of the pregnancy could be demonstrated was 1/10 in the recalcification system As mentioned addition of normal plasma to the patient s plasma did not shorten the prolonged coagulation times. Two-stage assays for inhibitors against factors VIII IX XI and XII showed no signs of an anticoagulant active against these factors. The effect of adding various amounts of human brain thromboplastin to the patient s plasma was investigated (Table III) The patient had a prolonged one-stage prothrombin time which became more pronounced when the thromboplastin was diluted In the presence of undiluted thromboplastin the patient s plasma (3/73) prolonged the clotting time of normal plasma by 1–2 sec in dilution 1/1 with thromboplastin in dilution 1/10 by 5–10 sec in dilution 1/2 with thromboplastin in dilution 1/100 by 43 sec in dilution 1/5 with thromboplastin in dilution 1/1 000 by 40 sec in dilution 1/10 For comparison thromboplastin titration was performed on plasma from one patient with severe haemophilia A (<1% factor VIII) and on plasma from one patient with severe haemophilia A complicated by anti-AHF Although the thromboplastin times were prolonged when thromboplastin was diluted, the changes were relatively small compared with those observed in the patient. The Stypven time was prolonged. The patient s plasma in dilution 1/2 prolonged the Stypven time of normal plasma from 20 to 29 sec It was also shown that intrinsic thromboplastin obtained from normal reagents lost its activity if the patient s citrated plasma was used as a test substrate As pointed out, the factor V and P&P levels were nor

mal She had no sign of a defect i the latest stage of the coagulation process and had a normal antithrombin III level Taken together these observations indicate that the anticoagulant in this patient inhibited the conversion of prothrombin to thrombin and was probably directed against the action of both extrinsic and intrinsic thromboplastin

The anticoagulant was demonstrated not only in plasma, but also in serum The anticoagulant was not removed from plasma by adsorption with BaSO or dialysis across a semipermeable membrane for 24 hours. The activity was unaffected by heating at 56°C for 30 min. When nomal plasma and patient s plasma were mixed the anticoagulant activity was demonstrable already after 3 min.

The anticoagulant was found to be neutralized by antihuman IgG antiserum when tested according to Feinstein et al (8)

DISCUSSION

The patient was a woman who had had three earlier pregnancies all of which had for some unknown reason terminated in intrauterine death. During the fourth pregnancy Rh immunization against c was observed. During the first and second pregnancies no such immunization could be demonstrated and during the third it was barely demonstrable There is therefore no reason to suppose that Rh immunization played any role in the cause of intrauterine death in the first three pregnancies

During the third pregnancy a coagulation defect was diagnosed which was characterized by prolonged coagulation times and prolonged one-stage prothrombin time. At examination between the third and fourth pregnancy the coagulation status was normal During the fourth pregnancy the coagulation time as well as the one-stage prothrombin time was prolonged It was demonstrated that the patient had a circulating anticoagulant It did not inhibit factors VIII IX XI and XII In spite of the prolonged one-stage prothrombin time and the prolonged Stypven time the values found for the factors in the prothrombin complex and for factor V were normal The thrombin time fibrinogen level and antithrombin III level were normal No signs of abnormalities in the fibrinolytic system could be demonstrated. Addition of thromboplastin in decreasing concentration to plasma prolonged the clotting times much more than those of normal plasma haemophilia A plasma and haemophilia A plasma with an anticoagulant against factor VIII According to Margolius et al. (18) this is the pattern seen in patients with anticoagulants directed against the action of thromboplastin. The patient s plasma also inhibited intrinsic thromboplastin obtained from normal reagents

Taken together the observations concerning the mode of action of the inhibitor indicated that the inhibitor interfered with the acti ation of prothrombin to thrombin by thromboplastin and most probably acted as an antithromboplastin According to Deutsch (6) pathological inhibitors of blood coagulation may belong to two different categories according to their mode of action One group of anticoagulants reacts with one of the clotting factors stoichiometrically in a bimolecular reaction and inactivates the clotting factor concerned The other group interferes instantaneously in some unknown way with one of the later reaction steps in the activation of prothrombin without inactivating an individual clotting factor It is quite clear that the anticoagulant of our patient belonged to the latter group Deutsch (6) points out that patients with inhibitors of the second type rarely have bleeding symptoms Our patient showed no signs of increased bleeding tendency

Table III *Effect of varying mounts of human brain thromboplastin*

Source of plasma	Undiluted	1/10	1/100	1/1 000	1/10 000	Recalcification time
Normal	15	28	53	76	95	170
Patient plasma	*20*	*44*	*120*	*220*	*287*	*470*
Haemophilia A	15	31	59	101	19[illegible]	460
Haemophilia A anticoagulant against f VIII	15	31	64	106	207	600
Normal plasma patient plasma dil. 1/5	15	[illegible]9	90	150		245

Acquired circulating anticoagulants are inhibitors which have been characterized as immunoglobulins (? 6 7) As mentioned above they may appear in various immunological disorders The ANF titre was positive, but no signs of any underlying immunological disease of the patient could be demonstrated

Circulating antiocoagulants against factor VIII have been shown to be capable of appearing during pregnancy and to persist for a long time, causing severe haemorrhagic diathesis (23) In our patient the coagulation time was normal between the third and fourth pregnancy and the anticoagulant titre fell after the fourth pregnancy (Table 1). During the fourth pregnancy she was found to have also leucocyte agglutinating and lymphocytotoxic antibodies which were directed also against the husband s cells. This suggests that the pregnancy and the foetus probably owing to passage of cell fragments and thromboplastic substances to the mother s blood had induced the development of antibodies against the foetal tissues and acted as antithromboplastin in the coagulation process.

No signs of intravascular coagulation could be demonstrated which may be explained by the assumption that thromboplastic substances passing to the maternal blood stream were neutralized by anti romboplastin existing there This anticoagulant not as mentioned cause any bleeding. But it quite feasible that the passage of circulating nticoagulants to the foetal blood stream with reaction to the foetal thromboplastin may cause bleeding with intrauterine death as a consequence In view of the immunological situation demonstrated the foetus may thus be regarded as an incompatible transplant It is very probable that a varying degree of incompatibility was present during the various pregnancies In the last pregnancy the incompatibility was obviously not more severe than that the child could survive But there were signs of placental insufficiency in the form of low excretion of oestriol in the urine and bi parietal measurements with ultrasonics showed retarded growth Owing to the laboratory findings and the observations made in the three earlier pregnancies the child was delivered by caesarean section in the 34th–35th week. The placenta was also full of infarcts and showed signs of bleedings and necrosis.

It is possible that in a case like this immunosuppressive treatment should be considered The favourable effect of such treatment has been shown in cases with circulating anticoagulants against factor VIII (10 17) and factor IX (20).

As far as we know this is the first time that intrauterine death has been shown to be a consequence of the formation of antibodies acting as antithromboplastin It shows that in women who have had a pregnancy terminating in unexplainable intrauterine death coagulation analysis is indicated with a special search for circulating anticoagulants

ACKNOWLEDGEMENTS

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Table I *Clinical and laboratory data on the patients in groups* ‿ *and D*

Ep=total erythropoiesis, figures within parentheses indicate megaloblastic cells Mp=myelopoiesis Nmc=non-myeloic cells (lymphocytes, plasma cells reticulum cells) Not inv =not investigated N=normal, + palpable on inspiration ++ easily palpable, +++ grossly enlarged

Pat. no.	Sex	Age (y)	Weight (kg)	Hb (g/l)	MCV (fl)	MCHC (g/l)	Reticulocytes (10^9/µl)	Bone marrow picture (%)	Blood smears	Spleen Size	Myel. metapl	Diagnosis
Group A												
1	♀	15	56	78	122	328	125	70 Ep 8 Mp 10 Nmc	Anisocytosis hypochromia, basophilic stippling	N	Not in	Paroxysmal nocturnal hemoglobinuria
2	♂	35	66	130	82	370	165	Not inv	Spherocytosis	(+)	Not inv	Hereditary spherocytosis
3	♀	29	65	104	76	330	50	42 Ep 15 Mp 15 Nmc	Spherocytosis	Splenectomized		Hereditary spherocytosis and iron deficiency
4	♂	23	52	81	109	320	480	86 Ep Mp 5 Nmc	Anisocytosis, polychromasia microspherocytosis	+	Not i	Autoimmune hemolytic anemia
5	♀	38	53	96	123	330	600	58 Ep 19 Mp 10 Nmc	Anisocytosis, polychromasia, basoph stippl. nucl red cells	Splenectomized		Hemolytic anemia (PNH like syndr with morning *hemoglobinuria* and hemosiderinuria)
6	♀	44	55	139	105	320	700	55 Ep 13 Mp 11 Nmc	Polychromasia, nucleated red cells	+	Not inv	Hemoglobinopathy (16)
7	♂	32	65	159	102	350	60	26 Ep 20 Mp 18 Nmc	Spherocytosis	+	Not inv	Hereditary spherocytosi
8	♂	68	69	73	112	340	290	35 Ep 20 Mp Nmc	Marked aniso- and poikilocytosis	++	+	Chronic myelogenous leukemia with secondary hemolytic anemia
Group B												
9 (I)	♀	40	67	104	117	330	70	10 Ep 35 Mp 15 Nmc	Some basophilic stippling	N	Not inv	Erythroblastopenia, on androgens, o ymetholone, 100 mg/day
9 (II)			68	97	114	300	63	10 Ep 30 Mp 77 Nmc	Normal			Erythroblastopenia on androgens, oxymetholone, 50 mg/day
10 (I)	♂	27	68	57	116	320	40	14 Ep 4 Mp 66 Nmc	Normal	N	Not inv	Aplastic anemia no treatment
10 (II)			68	70	85	320	9	Not inv	Normal			On androgens oxyme-

11	♀	76	55	94	90	320	5	9 Ep 40 Mp 14 Nmc	Normal	(+)	0 hemo-siderosis	plastic anemia no treatment
12	♂	76	63	89	94	360	21	14 Ep 19 Mp 28 Nmc	Some anisocytosis	N	Not inv	Pituitary adenoma, sec hypothyreosis no substitution
13	♀	20	49	119	101	320	70	31 (6) Ep 70 Mp 70 Nmc	Some anisocytosis	N	Not inv	Fanconi constitutional aplastic anemia. In remission on androgens xymetholone 100 mg/day
14	♀	63	67	83	113	330	85	60 Ep 21 Mp 6 Nmc	Anisocytosis poikilocytosis polychromasia	N	Not inv	Temporary bone marrow failure with pancytopenia after busulfan due t polycythemia rubra vera. M laena.
15	♂	65	71	84	94	370	15	Dry tap	Normal	N	Not in	Aplastic anemia, no treatment
Group C												
16	♀	68	64	85	97	310	51	36 (6) Ep 16 Mp 6 Nmc	Marked aniso-poikilocytosis, polychromasia	++	Not inv	Sideroblastic anemia
17	♂	68	58	93	95	310	32	50 (15) Ep 10 Mp 14 Nmc	Marked aniso-poikilocytosis polychromasia	N	Not inv	Sideroblastic anemia developing nto acute myeloic leukemia
18	♀	78	45	77	108	330	51	80 Ep 15 Mp 0.5 Nmc	Marked aniso-poikilocytosis polychromasia	N	Not inv	Sideroblastic anemia
19	♂	68	50	81	111	330	37	70 Ep 4 Mp (blast) 13 Nmc	Marked aniso-poikilocytosis polychromasia	++	Not	Acute myeloic leukemia
20	♂	70	99	60	170	364	18	48 (13) Ep 15 Mp 6 Nmc	Moderate aniso-poikilocytosis	+	Erythrocyto-phagocytosis some erythrop. metapl.	Chron. glomerulonephr + Liver cirrhosis. Treated with Cyclophosphamide and prednisolone
1	♂	56	86	67	93	300	81	54 (2) Ep 17 Mp 8 Nmc	Marked aniso-poikilocytosis, polychromasia		Not inv	Sideroblastic anemia finally developing to acute myel leukemia
22	♂	13	62	112	108	340	4	36 (14) Ep 21 Mp 10 Nmc	Moderate aniso-cytosis	(+)	Not in	Pern anemia in partial remission
3	♀	54	8	102	102	290	14	50 (22) Ep 13 Mp 8 Nmc	Moderate aniso-cytosis	N	Not inv	Sideroblastic anemia probably developing into acute leukemia

(Continuation on next page)

Table I *(cont.)*

Pat. no	Sex	Age (y)	Weight (kg)	Hb (g/l)	MCV (fl)	MCHC (g/l)	Reticulocytes ($10^9/\mu l$)	Bone marrow picture (%)	Blood smears	Spleen Size	Myel metapl	Diagnosis
24	♂	69	66	72	110	310	15	35 (3) Ep 40 Mp (mostly blast forms) 13 Nmc	Ess normal	N	Not inv	Refractory anemia developing into acute myeloic leukemia
25	♀	54	54	105	84	310	45	70 Ep 28 Mp 18 Nmc	Moderate aniso-poikilocytosis	++	Not inv	Sideroblastic anemia developing into acute myeloic leukemia
Group D												
26	♀	73	51	104	90	300	9	25 Ep 26 Mp 4 Nmc	Leukoerythrobl.	+	Slight	Myeloid metapl.
27	♀	53	68	94	100	364	48	9 Ep 8 Mp 24 Nmc	Leukoerythrobl.	++	Slight	Myeloid metapl
28	♂	67	66	81	84	290	90	53 Ep 76 Mp 1 Nmc	Leukoerythrobl.	++	Marked	Myeloid metapl.
29	♂	65	66	57	91	320	30	14 Ep 13 Mp 6 Nmc	Leukoerythrobl	+	Not inv	Myeloid metapl.
30	♂	72	74	87	89	320	30	43 Ep 70 Mp 8 Nmc	Leukoerythrobl.	+++	Slight	Myeloid metapl.
31	♂	70	71	105	89	320	77	65 Ep 11 Mp 5 Nmc	Leukoerythrobl.	++	Moderate	Myeloid metapl.
32	♂	66	80	139	84	370	13	42 (18) Ep 15 Mp 35 Nmc	Normal	+++	None	Splenic lymphoma developing into chronic lymphatic leukemia

splenomegaly six of whom had myelofibrosis (myeloid metaplasia) and one had malignant lymphoma restricted to the spleen. All patients were clinically stable and thought to be in steady state hematologically at the time of the study. Pertinent clinical and laboratory data are given in Table I.

METHODS

VCO was measured with closed rebreathing system according to the principles laid down by Coburn et al. (5, 6). After an equilibration period of 20 min blood samples were drawn every 15 min through an indwelling venous catheter. After 120 min (9 samples) 70.0 ml 99.9% pure CO (AB Alfax, Malmö, Sweden) were injected into the system and final blood sample was drawn after another 25 min. The CO content of the blood samples was determined by gas chromatography as described by Collison et al. (7, 24) modified as described by Landh et al. (19). 99.9% pure CO quantitated as described by Celegin et al. (3), was used for calibration. The Hb concentration was measured according to van Kampen and Zijlstra (17). The absolute VCO (ml/h STPD) was calculated from the formula

VCO = ΔCOHb · dilution factor

where ΔCOHb is the increase in blood COHb level per hour determined from the COHb of the first 9 blood samples, using the least square criterion to compute a best fit for the relation between time and COHb. The dilution factor was determined from the increase in COHb ($\Delta COHb_{inj}$) after the injection of known amount of CO (CO_{inj}) into the rebreathing system. The total CO binding capacity was calculated from the dilution factor

$$\text{CO binding capacity} = \frac{CO_{inj} \cdot 100}{\Delta COHb_{inj}}$$

Total body hemoglobin (TBHb, g) was obtained by dividing the CO binding capacity by 1.39 and the total body heme (TBH, mmol) by dividing TBHb by 16.1. The results of the VCO determinations are given in µmol/day and in µmol/mmol TBH and day. The S.D. for single analyses of COHb calculated from duplicate determinations on 100 samples with COHb varying between 0.46 and 4.61% was found to correspond to coefficient of variation of 3%. Duplicate analyses were always performed. The variation of VCO from month to month within the same individual was calculated from 20 determinations in seven healthy males with mean VCO of 1.3 µmol CO/mmol TBH and day. The S.D. from month to month for each individual was found to be 2.85 µmol, corresponding to coefficient of variation of 23%. The coefficient of variation for the regression coefficient used for the calculation of VCO was found to be 18% in the controls and 1 % in the 37 patients.

Normal values. VCO measured in 20 young healthy males fasting in the morning after at least 36 hours free from CO exposure was found to be 0.55±0.3 (mean ± S.D.) ml/h, corresponding to 585±340 µmol CO/day. Calculated in µmol CO/mmol TBH and day the VCO in this group was found to be 10.8±5.5 (range 4.0–16.0). The initial baseline COHb in this group was 0.45±0.20% (range 0.30–0.64) and the TBH 53.5±14.8 mmol, corresponding to 1...0±1.8 g Hb/kg b.wt.

Labelling of erythrocytes with ^{51}Cr was carried out according to method C from the ICSH Panel on Diagnostic Applications of Radioisotopes in Hematology (15). After correction for the elution of chromium according to the above mentioned recommendations, the data were plotted in linear diagram. The red cell survival was calculated from straight line fitted to all the data points. If the fit was not satisfactory the survival was calculated from a straight line through the first few points only.

Vheme-c was obtained by dividing total circulating Hb in mmol (obtained from the dilution of the ^{51}Cr-labelled red cells) by the red cell survival time (days).

Routine hematology was performed with the aid of Coulter-Counter type S. Reticulocytes were counted according to Björkman (1) and serum bilirubin was measured according to Gambino and Schreiber (11). Bone marrow smears were usually obtained by sternal puncture and stained with May-Grünwald-Giemsa. Iron staining was performed according to Weinfeld and Hansen (28).

RESULTS

Group A

All patients in this group were known to have hemolytic anemia. The bone marrow picture was dominated by normoblasts and the reticulocyte count was elevated (Table I). All these patients had a high VCO (Table II) absolutely (µmol/day) as well as relatively (µmol/mmol TBH and day). In six patients the VCO was compared to Vheme-c calculated from the red cell survival and large variations were found. In two patients, patient 1 with a firm diagnosis of PNH and patient 5 who fulfilled some of the PNH criteria, the VCO was much less than the Vheme-c suggesting heme losses or heme catabolism without corresponding CO formation. In patient 3 splenectomized due to hereditary spherocytosis and now iron deficient due to severe menorrhagia and with only a moderate decrease in red cell survival and in patient 6 whose hemolytic anemia was caused by a hemoglobinopathy the VCO/Vheme-c quotient was around 1.3. In patient 8 an elderly male with severe Coombs test negative hemolytic anemia added to the final stages of a chronic myelogenous leukemia, the VCO was found to be almost double the value derived from erythrocyte turnover (VCO/Vheme-c 1.87). Patient 7 with *hereditary spherocytosis, was found to have a similar* increase in VCO compared to red cell survival (VCO/Vheme-c 1.76).

Table II *Carbon monoxide production and hemoglobin catabolism*

Pat. no.	TBHb ^{51}Cr (mmol heme)	TBH CO dilution (mmol)	Red cell Hf span ^{51}Cr (d.)	Vheme-c (µmol/d.)	VCO µmol/d	VCO µmol/mmol TBH and d.	VCO/Vheme-c quotient	Serum bilirubin (µg/ml)	COHb (Initial sample)
Group A									
1	14 1	16.3	17	835	510	31 6	0 61	10.0	1 05
2	25 6	30.4	30[b]	850	1 390	45 7	1 68	8 0	0.90
3	27.8	30 0	60	470	608	20.2	1.30	13 0	0.99
4	17.3	21 7	9	1 700[a, b]	1 600	74 0	0 94	49 0	1 70
5	20.2	21.5	9	2 220	1 650	77 0	0.74	25.0	1 70
6	34 0	37 7	17	2 040	2 795	83.2	1.37	60 0	1 70
7	50.8	53.6	70	725	1 280	23.8	1 76	15 0	1.27
8	27.8	27 9	39	715	1 320	44.8	1.82	13 0	1.50
Group B									
9 (I)	22.9	29 0	68	317	394	13 4	1 17	7 0	0.65
9 (II)	24.6	33.2	93	266	480	13 6	1.80	8.0	0 44
10 (I)	12.4	21.0	125	100	181	8.9	1.81	3.0	0.46
10 (II)	Not inv	21.6	Not inv	–	490	21.6	(4 90)	–	0 61
11	19 7	24.5	37	530	610	24.5	1 15	5 0	1 09
12	20.3	27 1	145	135	234	8.6	1.34	4.0	0.54
13	19.3	23 9	89	213	618	25.8	2.90	9 0	0.76
14	30.1	29 0	49	620	383	13 1	0.62	7.0	0 77
15	17.8	20.7	51	356	555	26 7	1.56	4 0	0 65
Group C									
16	18 1	28 6	70	290	745	28.6	2.98	9 0	0 70
17	22..	29.2	64	345	820	27.8	2.38	8.0	0 77
18	12.4	18.5	73	174	468	25.2	2.69	8 0	0.88
19	24.9	26 1	65	374	975	38.0	2.60	4.0	1 15
20	21 4	29 9	22	970	2 450	82.0	2.51	19 0	1.57
21	20.4	25.3	33	612	1 570	60.2	2.56	13.0	1.25
22	39 0	46.5	136	290	770	17.2	2.66	15 0	0.66
	23 9	33 1	67	358	995	30.0	2.78	9 0	0.87
	21 0	24 8	100	210	586	24.2	2.80	4 0	0.81
	21 7	25 9	71	304	715	27.6	2.37	7 0	0.68
Group D									
26	19 4	22 9	88	213	352	15 4	1 65	5 0	0 47
27	22.6	36.4	39	590	1 240 (1 050)	34 6	..10 (1 78)	8.0	0 62
28	30.8	43 0[c]	30	1 050	2 180 (1 850)	50 0	2 04 (1 76)	8.0	1 70
29	15.2	14 4	44	350	533 (615)	37.2	1.62 (1 75)	11.0	0 72
30	56.0	59.5	68	840	1 130	19 0	1.35	7.0	0 72
31	29.0	30.8	25	1 265	1 655	53.8	1.31	8.0	1 90
32	68.1	79.8	97	704	892	11.2	1.27	8 0	0.64

Estimated from TBH (CO dilution) Fig. 4 Calculated from the reticulocyte count. Probably erroneously high or [d] low values. Figures within parentheses = corrected values.

Group B

The patients in this group were characterized by a hypoproliferative anemia and all except patient 14 had hypo- or acellular bone marrow pictures (Table I). The absolute VCO in these patients was usually low (Table II). In relation to TBH and day VCO was normal or moderately increased In four patients the VCO/Vheme-c quotient varied between 1 15 and 1.56 while in three the quotient was high (≥1.8) suggesting an increase in extraerythrocytic CO production. Two of the latter patients (no 9 with erythroblastopenia and no. 13 with Fanconi's constitutional aplastic anemia) were on pharmacological doses of androgens while the third (no 10 study I) a young male with idiopathic aplastic anemia was not receiving any form of

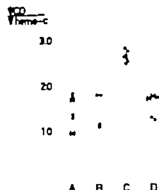


Fig. 1. VCO/Vheme-c ratio in 8 patients with hemolysis (group A), 7 patients (one of whom was studied twice) with hypo- or aplastic bone marrow (group B), 10 patients with refractory anemia and hypercellular bone marrow (group C), and 7 patients with splenomegaly (6 of whom had myeloid metaplasia) (group D). Circles within parentheses in group A indicate ratios where Vheme-c was estimated from the reticulocyte count and in group D ratios corrected for probably erroneous TBH values. × = patients on pharmacological doses of androgens.

treatment. In patient 14 the VCO/Vheme-c quotient was very low (0.62), suggesting some heme loss (catabolism) without CO formation, an assumption corroborated by the finding of a continuous melaena secondary to thrombocytopenia.

Group C

This group consists of ten patients with anemia in combination with a hypercellular bone marrow dominated by erythropoietic, sometimes megaloblastic, cells and a low reticulocyte count (Table I). Absolute VCO was increased in all patients and the values were sometimes very high. One of the highest values of the study was seen in patient 20, a young boy with chronic glomerulonephritis and liver cirrhosis with splenomegaly, treated with cyclophosphamide and prednisolone. In addition to the high cellularity his bone marrow picture was characterized by a marked erythrocytophagocytosis. Even though red cell survival was short, 22 days, heme catabolism calculated from red cell survival represented only 40 % of total heme catabolism (VCO/Vheme-c quotient 2.5) in this case. Since average red cell survival in this group was only moderately shortened, the high VCO resulted in VCO/Vheme-c quotients between 1.5 and 3.0, suggesting a marked increase in CO production unrelated to circulating red cells in all cases in this group (Fig. 1).

Group D

This group consists of seven patients with splenomegaly, six of whom had myelofibrosis (myeloid metaplasia) and one a malignant lymphoma restricted to the spleen. The diagnosis of myelofibrosis was supported by findings of a hypoproliferative anemia with splenomegaly and myeloid metaplasia in liver and spleen and a leukoerythroblastic peripheral blood picture. Absolute VCO varied from a low value (patient 26) to very high values (patients 28 and 31). There were, however, large variations in the red cell survival too, from an essentially normal value (patient 26) to rather short values (patients 28 and 31). When CO production and red cell survival were compared in the VCO/Vheme-c quotient, two patients (nos. 27 and 28) were found to have high values, 2.10 and 2.04, which should indicate an increase in extraerythrocytic heme catabolism. However, when compared to body weight and Hb concentration, the TBH determinations in these two patients seem to have given erroneously high values. Since an erroneous TBH value will result in a falsely absolute VCO—whereas the relative VCO (μmol/mmol TBH and day) will not be affected—the VCO/Vheme-c quotients in these two patients may be misleadingly high. In a third patient (no. 29) the

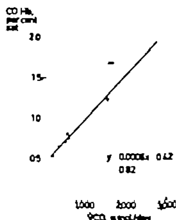


Fig. 2. Comparison between the COHb in the initial blood sample and the absolute VCO (μmol/day) in 32 patients with hematological disorders. Two patients were studied twice.

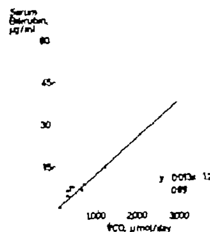


Fig 3 Comparison between the concentration of unconjugated serum bilirubin and the VCO (µmol/day) in 31 patients with hematological disorders. One patient was studied twice.

TBH seems to be falsely low In the patient with malignant lymphoma restricted to the spleen which weighed 6 kg at splenectomy the VCO/Vheme-c quotient was close to unity

General

The relation between baseline COHb and absolute VCO (µmol/day) is seen in Fig 2 The correlation is ighly significant ($r=0.82$ $n=34$) There was also a ificant correlation between the concentration of ... reacting bilirubin and absolute VCO 0 69 $n=34$) (Fig. 3) A comparison was also made between TBH measured with CO dilution technique and total circulating Hb mass measured after labelling red cells with Cr (Fig. 4) The linear regression of this relationship follows the equation

TBH mmol heme=(1 02 TBHb+4 35) mmol heme

with a correlation coefficient of 0 97 Three determinations with the CO technique were probably not correct (see Results group D) and are not included in the calculation

DISCUSSION

The original studies on VCO (5 6) suggested only small inter and intraindividual variations but further investigation has not corroborated these findings (21). Consecutive studies on some of the young men forming the present control group verified a large variation within the same individual from week to week. More than 50% of this variation is due to the rather large error in the measurement of VCO However when expressed as a percentage of the regression coefficient the error is inversely proportional to VCO and is usually around 10% when the relative VCO (µmol/mmol TBH and day) is increased by 100% of the normal or more which was the case in 27 of the 32 patients in the present study

Measuring red cell survival with the aid of labelling erythrocytes with Cr is hampered by the fact that the isotope disappears from the blood not only through the demise of the erythrocytes but also through elution from the cell Two main problems arise: firstly there may be individual variations in the elution rate (4) secondly this elution will affect the accuracy of the measurement mainly when red cell survival is normal or slightly decreased. When VCO is compared with data on the daily turnover of red cell hemoglobin (V̇heme-c) calculated from red cell survival allowance must be made for the fact that VCO represents the heme catabolism of the hours during which the study is performed while the estimation of red cell survival is based upon observations made over several weeks

Another reservation concerns the difficulty in obtaining uniform groups of patients. A common diagnosis does not guarantee that the patients will yield similar data in all variables The only way

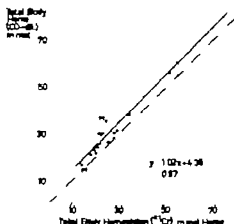


Fig 4 Comparison between simultaneous measurements of TBH estimated with the CO dilution method, and the circulating TBHb estimated with the Cr dilution method in 30 patients with hematological disorders. Circles within parentheses are not included in the calculation of the regression line, since when compared to body weight and Hb concentration the TBH value was thought to be unreliable. One patient was studied twice.
-- identity line, — =regression line

round this problem is to present a lot of data on each patient, so that the reader may form an opinion by himself

Studying a group of normal males Coburn et al (6) found the mean VCO/V̇heme-c ratio to be 1.27 Even though this figure is calculated from a small material it seems reasonable that total heme catabolism should exceed catabolism of red cell Hb by 30% The true figure may be even higher since Coburn s group used the TBH value obtained by CO dilution and not the TBHb obtained by ^{51}Cr dilution. Correcting for this difference, the VCO/Vheme-c ratio in Coburn s material becomes 1 44 Although we have no isotope red cell survival studies in our control group it can be shown indirectly that the figure given must be fairly correct. In our control group the mean TBH was 53 4 mmol. From the regression line in Fig. 4 it will be found that this corresponds to 48 mmol circulating Hb heme. A normal turnover of this heme will cause the formation of 48 000/120 = 400 µmol CO/day The mean daily absolute V̇CO in the group was 590 µmol corresponding to a V̇CO/Vheme-c ratio of 1 48. Accordingly a third of the daily heme turnover must derive from sources other than circulating red cells. In hemolytic anemia of not too complex nature as in hereditary spherocytosis the figure might be smaller because the portion corresponding to the turnover of red cells increases substantially Even though it is known that the "early labelled" peak of bilirubin is increased after phlebotomy and in hemolysis (23) it has not been shown that the relation between effective and ineffective erythropoiesis is changed in the direction of a higher degree of ineffective erythropoiesis Coburn et al. (6) calculated the VCO/Vheme-c ratio in seven patients with hemolytic anemia and found it to be 1 40 not significantly different from their reference value of 1.27 and close to our calculated reference value of 1 5

The patients in group A are not a uniform group as can be seen from the diagnostic data in Table I. *This may explain the variations in the VCO/* Vheme-c ratio in this group Two values were clearly below unity indicating heme losses without CO formation. One was from patient 1 with PNH The intravascular hemolysis characterizing this disease is known to result in heme losses via the urine (14), which might result in a low VCO/Vheme-c ratio. The other low value was found in a female patient (no. 5) studied on many occasions during the last 20 years in various hospitals without demonstration of any hemoglobinopathy enzyme deficiency or membrane abnormality However she had morning hemoglobinuria and increased amounts of hemosiderin in her urine and she may represent a partial PNH syndrome even though many of the PNH tests (Ham s Crosby s, and inulin tests) were negative. In patients 2 and 4 red cell survival studies were not performed. However daily turnover of red cells can be estimated at least semiquantitatively from the reticulocyte count corrected according to Finch (10) assuming that the patients are in steady state It will then be found that the daily Hb heme turnover represented 4 and 10% which gives V̇CO/Vheme-c ratios of 1 7 and 0.94 respectively Consequently even though the range is wide the average ratio among the patients with hemolytic anemia is around 1.5 the calculated normal mean value

The patients in group B had in common a *hypoproliferative anemia with hypo- or acellular* bone marrow pictures and pancytopenia. The majority had a VCO/Vheme-c ratio around the calculated normal value of 1 5 (1 15–1.80). The very low value comes from a patient with pancytopenia and continuous melaena through the study period which explains her shortened red cell survival without any simultaneous increase in CO formation. The very high value 2.90 is from female (patient 13) with Fanconi s constitutional aplastic anemia, given oxymetholone 150 mg daily Two other patients (no 9 studies I and II and no 10 study II) were also on pharmacological doses of this drug. Even though the second study of patient 10 was not accompanied by a red cell survival study it can be seen that the VCO/Vheme-c ratio in patients given oxymetholone is within the calculated normal range or higher There are no signs of any substantial decrease in VCO during oxymetholone therapy as was suggested by Lundh et al (20) in studies of the VCO/Vheme-c ratio in patients with sickle cell anemia given androgens in pharmacological doses. *The difference might be due to* the fact that the sickle cell anemia patients were given testosterone enanthate, but it is more probable that the assumption was based on too small a material If testosterone has any effect on the VCO/Vheme-c ratio, it should be an increase since testosterone is known to induce the synthesis of short-lived heme-containing enzymes in the liver (1).

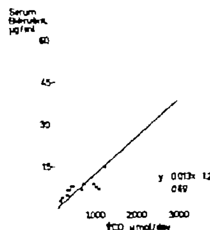


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The original studies on VCO (5 6) suggested only small inter and intraindividual variations but further investigation has not corroborated these findings (71) Consecutive studies on some of the young men forming the present control group verified a large variation within the same individual from week to week. More than 50% of this variation is due to the rather large error in the measurement of VCO However when expressed as a percentage of the regression coefficient, the error is inversely proportional to VCO and is usually around 10% when the relative VCO (µmol/mmol TBH and day) is increased by 100% of the normal or more which was the case in 27 of the 32 patients in the present study

Measuring red cell survival with the aid of labeling erythrocytes with [51]Cr is hampered by the fact that the isotope disappears from the blood not only through the demise of the erythrocytes but also through elution from the cell Two main problems arise firstly there may be individual variations in the elution rate (4) secondly this elution will affect the accuracy of the measurement mainly when red cell survival is normal or slightly decreased When VCO is compared with data on the daily turnover of red cell hemoglobin (Vheme-c) calculated from red cell survival allowance must be made for the fact that VCO represents the heme catabolism of the hours during which the study is performed, while the estimation of red cell survival is based upon observations made over several weeks

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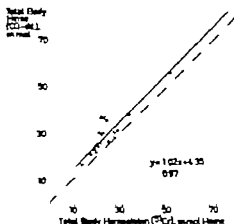


Fig 4 Comparison between simultaneous measurements of TBH estimated with the CO dilution method, and the circulating TBHb estimated with the [51]Cr dilution method in 30 patients with hematological disorders. Circles within parentheses are not included in the calculation of the regression line, since when compared to body weight and Hb concentration the TBH value was thought to be unreliable. One patient was studied twice. –=identity line — =regression line

ence is probably due to a higher degree of air pollution and CO contamination in urban areas of the USA. Accordingly if CO contamination can be excluded for more than 36 hours, the morning COHb will closely reflect total heme catabolism.

ACKNOWLEDGEMENTS

This investigation was supported by grants from the Swedish Medical Research Council (Projects Nos. 19X-3523 and 61P 3611), from the Medical Faculty University of Lund, from Selma and Augusta Svensson Fund for Cancer Research and from John and Augusta Persson Foundation for Scientific Research.

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NICOTINIC ACID AND THE ENDOGENOUS PRODUCTION OF CARBON MONOXIDE

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Abstract The endogenous production of carbon monoxide (VCO) has been followed with the aid of a rebreathing system for 3 hours in four healthy volunteers after injection of 50 mg nicotinic acid (NA) After an initial slight decrease for 15–30 min in the CO hemoglobin per cent saturation (COHb) rapid increase was registered for 170 min, whereafter the VCO returned to the normal, preinjection level The amount of "extra" CO produced varied between 4 1 and ... ml corresponding to 9 and 1 6 g Hb or 182 and 98 µmol heme, respectively These figures are 3–5 times higher than those reported in the literature calculated from increases in serum iron, bilirubin and COHb (without the use of rebreathing system). When related to the total body heme (TBH) estimated with the CO dilution technique the amount of extra heme metabolized after NA corresponded to 0.30% (range 0 26–0 32) of the TBH (a fourth of the total daily heme turnover or third of the daily Hb heme catabolism)

When Mattei (1) in 1946 reported that nicotinic acid (NA test) gave rise to a temporary hyperbilirubinemia, he suggested that the effect was due to an increase in heme catabolism. This hypothesis was verified by Laurell (8) and Gydell (5 6) who demonstrated a concomitant increase in serum iron and in carbon monoxide hemoglobin per cent saturation (COHb), respectively The absolute amount of heme turned over in the NA test has not been known for certain. From calculations based on changes in serum bilirubin and serum iron and simultaneous determination of plasma volume, Gydell (5) calculated the amount of extra Hb broken down to 0.5 g, corresponding to twofold increase in normal Hb turnover for two hours

A rebreathing system enables one to directly follow the amount of heme catabolized since all CO formed will be trapped and bound to the CO-binding pools in the body Assuming that these pools are in equilibrium, measurement of changes in COHb should give an accurate estimate of the speed and the absolute amount of extra heme turnover in the NA test.

MATERIAL AND METHODS

Two healthy males aged 31 and 40 years and two healthy females, aged 31 and 35 years well informed about the objectives and means of the investigation volunteered for the study They were known to be free from any hematological or hepatobiliary disease

The endogenous production of carbon monoxide VCO was measured according to Coburn et al ( ) All studies were performed with the subject fasting between 8 00 and 11 00 a.m. Three of the subjects had not smoked for at least three years and the fourth (no 4) smoked 10–15 cigarettes daily but abstained for 36 hours before the VCO study

After 20 min equilibration period in the rebreathing system, blood samples were drawn every 1 min through an indwelling venous catheter Immediately after the fourth sample had been drawn, 50 mg NA was given over period of 30 sec Blood samples were then drawn for altogether 255 min (18 samples) Every 30 min another sample of 10 ml was drawn for determination of bilirubin Immediately after the NA injection the subject experienced burning sensation in the skin especially in the face This sensation subsided within few minutes No other side-effects were noted

The CO content of the blood was measured with gas chromatography as described by Rodkey et al 3 14) modified as described by Lundh et al 10) The Hb concentration was measured according to van Kampen and Zijlstra (7) and bilirubin according to Michaelsson et al (13) The CO binding capacity (CO B C) was measured as described by Lundh et al (10) in at least three separate determinations on each subject Simultaneously the baseline production of CO (VCO_{basal}) was measured. The mean value of these three determinations will be used in the calculations in the present study Knowing the CO B C changes in COHb over certain period (ΔCOHb) can be used to calculate the VCO for this period using the formula

VCO ml = ΔCOHb CO B C ml

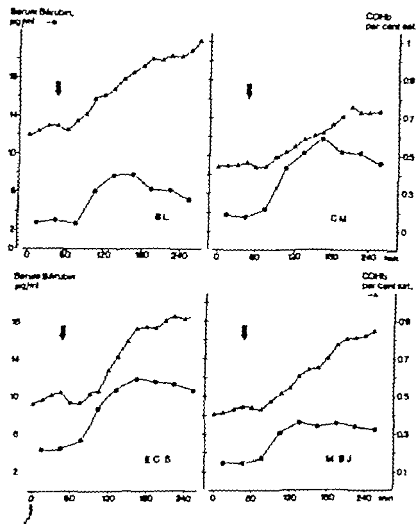


Fig. 1. Changes in COHb and serum bilirubin in four healthy volunteers after the NA test (arrow). The volunteers were kept in a rebreathing system throughout the study.

Furthermore, CO B C can be converted into total body Hb (TBHb) after dividing by 1.39 which in turn can be converted into mmol total body heme (TBH) after dividing by 16.1. The VCO related to NA (VCO_{NA}) was calculated from the total VCO during the period following NA injection (VCO_{total}) after subtracting the mean baseline VCO measured in at least three separate studies ($VCO_{baseline}$).

RESULTS

As will be seen from Fig. 1 the NA test resulted in marked changes in COHb in all subjects studied. Immediately after the injection there was a slight decrease varying between 0.01 and 0.06 COHb. The lowest point occurred at 15 or 30 min after the injection. There was then a rapid increase in COHb varying between 0.30 and 0.40 COHb. This rapid increase usually started before the 45th min after the injection and persisted for another 120 min after which COHb seemed to increase at the normal (baseline) speed indicating a return to a normal production of CO.

Serum bilirubin was measured twice before the injection of NA without showing any significant changes. After the injection an increase was measured after 30 min in three of the cases and the maximum level was reached after 120 min, when an almost threefold increase was demonstrated in all cases. No significant amounts of conjugated bilirubin were found in any of the samples taken. The absolute amount of "extra" heme catabolized after the NA test was calculated from the measurements of VCO (Table I). The "extra" CO produced after the NA test varied between 4.10 and 1.80 ml representing 2.91 and 1.29 g Hb which corresponds to 182 and 80 μmol heme respectively. The latter value, from subject 2, is probably falsely low and it will be seen from Fig. 1 that the COHb was constant in four samples before the injection and in the last

Table I *Carbon monoxid binding capacity (CO B C) total body heme (TBH) total endogenous produc tion of CO (VCO_{total}) baseline endogenous production of CO ($VCO_{baseline}$) and the ndogenous production of CO related to nicotinic acid ($\dot{V}CO_{NA}$)*

Case no.	Sex	Age (y)	B wt. (kg)	CO B C (ml)	TBH (mmol)	VCO_{total} (ml)	$VCO_{baseline}$ (ml)	VCO_{NA} (ml)	Extra heme catabolized µmol	Extra heme catabolized % of TBH	Extra Hb catabolized (g)
1	♂	40	82	1 270	56 8	6.03	1.93	4 10	182	0.32	91
2	♂	31	65	1 000	44 6	3.00	(1.20)	(1.80)	(80)	(0.18)	(1.29)
							0.00	3.00	134	0.30	2.13
3	♀	35	80	835	37.5	3 42	1.20	2.22	98	0.26	1 61
4	♀	31	54	730	32.7	3 43	1 04	2.39	106	0.32	1 71

Figures within parentheses, see Results

four samples This indicates that the *real* $VCO_{baseline}$ in subject 2 during the day of the NA test was not significantly different from zero Correcting for this VCO_{NA} in subject 2 becomes 3 0 ml corresponding to ~13 g Hb or 134 µmol heme When the extra heme catabolized is calculated as a percentage of the total body heme the values lie within the range of 0.26–0.32%

DISCUSSION

The main advantage with a rebreathing system in heme catabolism studies is that it allows one of the metabolites to be collected completely without seriously affecting the equilibrium of the system The error is rather large, around 20% for values of VCO within the normal range but it is much smaller 10% or less (9) when VCO is increased by 100% or more which seems to be the case in the NA test.

All subjects in the present study had a normal VCO measured in three separate studies The NA test caused an increase in serum bilirubin of the same degree as reported by Matter (12) Gydell (5 6) and Fromke and Miller (4) indicating that the data obtained from the CO studies may be used as a basis for calculating the amount of extra heme turned over in the NA test in normal man.

Even though the $\dot{V}CO_{baseline}$ was also calculated from the increase in COHb in the first four samples drawn before the injection we find it more correct to use the mean VCO obtained from three independent VCO studies performed under basal conditions especially as the study is then based on nine samples drawn during 120 min However this method was not used in our case 2 because his VCO on the day of the NA test did not differ significantly from zero a finding which serves to illustrate the large intraindividual day-to-day variation in VCO (9 11) The results for case 2 are therefore given with the $VCO_{baseline}$ assumed to be nil.

The results for the extra heme turned over in the NA test suggest an extra Hb turnover varying be tween 1 7 and 3.3 g. These are much higher values than those calculated by Gydell (5) from the increases in serum iron and serum bilirubin in the NA test and compared to the injection of non-viable red cells. However the fact that Gydell could not ex clude changes in the clearance of bilirubin and iron from plasma and did not follow his cases for more than 60 min might explain his much lower values On the other hand it is quite clear from our study as well as from that of Gydell (5) that the amount of heme broken down in the NA test is related to the total Hb mass The extra heme catabolized varied within a rather narrow range. 0.26–0.32% of TBH mean 0.30% These figures should be compared with the normal daily heme catabolism, which measured in our laboratory with the CO production technique amounts to 1 1% of the TBH pool in healthy young men. The extra heme metabolized in the NA test accordingly represents more than a fourth of the normal daily total heme turnover or a third of the daily turnover of circulating Hb heme. The use of a constant absolute dose of NA led to variations when the dose was calculated per kg body weight. However no relation was found between the dose (mg/kg) and th amount of extra heme catabolized.

The decrease in COHb in the first two samples after the injection of NA might represent changed equilibrium between the heme pools in the body elicited by the vasodilator effect of the drug. This effect might abate successively leading to a r

ration of the initial equilibrium something which has not been corrected for If corrected for the values for extra heme catabolized should be 5–10% smaller

The present study has not thrown any light on the mechanism behind the increased heme turnover induced by nicotinic acid. But we have shown that it is possible to directly measure the absolute amount of extra heme metabolized in the NA test and that this amount seems to be up to five times larger than suggested by earlier calculations from increases in serum iron and bilirubin.

ACKNOWLEDGEMENTS

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OXYGEN UPTAKE AND CARDIAC OUTPUT DURING SUBMAXIMAL AND MAXIMAL EXERCISE IN ADULT SUBJECTS WITH TOTALLY CORRECTED TETRALOGY OF FALLOT

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Abstract Ten female and eight male adults with tetralogy of Fallot, the majority totally corrected at adult age have been studied at rest and during submaximal and maximal exercise on bicycle ergometer Oxygen uptake was determined by the Douglas bag technique and cardiac output by the dye-dilution method. Maximal oxygen uptake was reduced about 30–40% from normal. Thus a complete normalization of the aerobic working capacity was not achieved in spite of an intracardiac repair that was considered surgically satisfactory Cardiac output response to exercise was subnormal, mainly due to small stroke volumes and partly because of low heart rates. A fall in stroke volume of more than 10 ml was found in 8 of the patients during exercise. N correlation was found between stroke volume during maximal exercise, on the one hand and the presence of particular residual defect, anatomy of the right ventricular outflow tract prior to operation and the use of right ventricular outflow patch on the other However too few patients were studied to allow any definite conclusions as to the possible influence of these variables. It remains to be shown whether the haemodynamic abnormalities will be less and the aerobic work capacity better if total correction is undertaken at an early age

The first patient with tetralogy of Fallot (TOF) was totally corrected in 1955 (32) Thus the knowledge of the long-term results is incomplete Total corrective operation has dramatically changed and improved the natural history for patients with TOF Still even a successful operation must be looked upon as an anatomical correction with some residua since the patients are left with a entriculotomy scar often with some degree of right ventricular outflow tract stenosis and a right bundle branch block (RBBB).

The question is whether these residua will interfere with the normalization of the circulation which is aimed at when total correction is undertaken and whether the impact of these residua is influenced by the age at which total correction is performed.

Several authors (9 17 18 33 40) ha e found normal or near normal haemodynamics at rest and during mild exercise in patients with technically good operation results However this does not mean that they have a normal exercise tolerance and a normal haemodynamic response to heavy work. In the haemodynamic studies cited above, most patients were corrected before the age of 10 The aim of this study was therefore to study the haemodynamic response during submaximal and maximal exercise in a group of patients with TOF corrected at adult or near adult age. The haemodynamic study presented in this article is part of a larger investigation comprising pulmonary and renal function as well as muscle metabolism in patients with TOF (5 7 8)

MATERIAL

Eighteen adults, 10 females and 8 males, with totally corrected TOF took part in this study All belonged to the material previously studied by Möller (35) and all had undergone palliative shunt operation between 1947 and 1955 at a mean age of 5 years. Total correction was undertaken at 19.5 years of age (range 11–27 5) Only 3 patients were less than 15 years old when intracardiac repair was performed. The mean age at the follow-up was 26 (range 19–35). Since total correction 6.5 years (range 2 5–10 5) had thus elapsed. The anatomy classification suggested by Hawe et al (19) was used for the right ventricular (RV) outflow tract before operation. Eight patients had isolated infundibular stenosis (type I) 7 combined valvular and infundibular stenosis (type II) and 3 diffuse hypoplasia of the outflow portion of the RV and the main pulmonary artery (type III). Intracardiac repair was carried out in the Departments of Thoracic Surgery Karolinska Sjukhuset, Stockholm (14 cases) and in the University Hospital,

Table I *Individual values for oxygen uptake (V_{O_2}) cardiac output ($\dot{Q}$) heart rate (HR) stroke volume (SV) and blood lactate concentration at rest (R) in supine position and during submaximal (E E_1) and maximal exercise (E_{max}) in the sitting position*

Pat. no	Age (y)	Sex	Height (cm)	Weight (kg)	Blood vol (l)	Exercise state	V_{O_2} (l/min)	$\dot{Q}$ (l/min)	HR (beats/min)	SV (ml)	Lactat (mmol/l)	Remarks[a]
1	28.5	♀	170	73.5	4 7	R	0.25	5 6	85	66	0.6	Type I
						E_1	0.97	10.1	118	85	[illegible]0	VSD (minimal)[b]
						E_4	1.57	13 0	152	85	4.2	
						E_{max}	1 95	15.5	174	89	11 1	
2	25	♀	164	55	4 1	R	0.21	4 4	74	60	0.5	Type I
						E	0.88	8.2	128	64	–	–
						E_4	1 02	9 1	152	60	4.8	
						E_{max}	1 18	9 4	170	55	7 0	
3	22	♀	167	60.3	4 4	R	0 21	4.5	63	72	0 7	Type II
						E_1	0.93	9 1	117	78	3.2	PI
						E_4	1.36	10 1	156	65	3 6	
						E_{max}	1 61	13 4	173	78	6.0	
4	22	♀	167	48 4	4 7	R	0.[illegible]1	3 9	66	59	1.5	Type II
						E_1	0 82	6.3	1[illegible]4	51	3.8	PI
						E_4	1 16	6 7	168	40	6.9	
						E_{max}	–	8 9	182	49	10 1	
5	27.5	♀	163	62.7	4.5	R	0 21	3 9	61	63	0 8	Type II
						E	0 84	7.3	99	73	5.3	PI
						E_4	1.33	8.5	145	58	5.[illegible]	
						E_{max}	1 48	9 1	178	51	10.8	
6	27	♀	163	48 4	4.2	R	0.25	4.3	71	61	0 6	Type I
						E_1	0.82	6.3	101	63	3	
						E_4	1 42	10 0	165	61	12.1	
						E_{max}	1.57	11 4	176	65	15 7	
7	23.5	♀	161	50.5	3.5	R	0.24	4 1	78	53	1 1	Type II
						E_1	0 61	6.5	121	53	3 0	PI
						E_4	0.85	7.3	149	49	4.8	
						E_{max}	1 08	7 9	171	46	9 7	
	35	♀	164	58		R	0.29	5 4	86	63	0.7	Type III
						E	0.96	10.7	121	88	3.2	PI (pronounced)
						E_4	1.26	11 0	143	77	5 9	+PS
						E_{max}	1.53	10 9	164	66	9 7	
9	24	♀	158	62	3.8	R	0.21	4 6	76	61	0 9	Type I
						E_1	–	7.3	109	67	5 0	VSD+PS+PI[b]
						E_4	1 16	9 0	139	65	7 3	
						E_{max}	1 53	9 7	174	56	15.6	
10	1	♀	165	65	3.8	R	0 19	4 4	83	53	0.8	Type II
						E_1	0.79	7 2	109	66	2.0	PI[b]
						E_4	1 27	9.5	143	66	3 1	
						E_{max}	1 68	11 7	186	63	7 9	
11	25	♂	189	67.5	6 3	R	–	6 3	79	80	1 0	Type III
						E_1	1 49	10.5	121	87	3.3	PS+PI+VSD
						E_4	1 97	12.5	150	83	5.8	(minimal)[b]
						E_{max}	2 34	13 4	177	76	12 8	
12	19	♂	168	67	5.0	R	0.26	5 3	66	81	1 1	Type I
						E_1	0 91	9.2	104	89	–	PS+VSD[b]
						E_4	1.86	12.3	155	79	3.7	
						E_{max}	[illegible].30	14 1	187	76	10.0	
13	25	♂	171	56.1	4.2	R	0 26	5 1	78	66	0.5	Type II[b]
						E_4	1 05	8.7	129	67	3 1	PS+PI
						E_1	1 44	11 4	168	68	6.0	
						E_{max}	1.66	12.4	185	67	14.0	

Table I (*cont.*)

Pat. no.	Age (y)	Sex	Height (cm)	Weight (kg)	Blood vol. (l)	Exercise state	$\dot{V}_{O_2}$ (l/min)	$\dot{Q}$ (l/min)	HR (beats/min)	SV (ml)	Lactate (mmol/l)	Remarks[a]
14	29	♂	180	79	3.5	R	0.37	4.7	55	86	0.6	Type I
						E_1	1.48	10.1	123	82	–	[illegible]
						E_4	1.90	17.5	156	80	3.9	
						E_{max}	[illegible]11	14.1	179	79	7.4	
15	25	♂	16[illegible]	61.2	3.8	R	0.[illegible]3	5.7	57	99	0.8	Type I
						E	0.88	9.0	84	108	2.1	VSD (minimal)[b]
						E_4	1.43	1[illegible]3	129	95	4.1	
						E_{max}	[illegible]01	13.5	174	78	9.0	
16	31	♂	192	106	6.4	R	0.33	4.3	6[illegible]	70	1.4	Type I
						E_1	1.39	11.[illegible]	106	106	3.0	
						E_4	2.04	14.8	145	102	6.5	
						E_{max}	[illegible]33	13.7	161	85	10.4	
17	26.5	♂	175	6[illegible]	4.9	R	0.29	6.4	75	85	0.9	Type II
						E_1	1.44	9.9	120	83	–	
						E_4	–	12.3	168	73	6.7	
						E_{max}	2.19	14.4	183	78	13.6	
18	30.5	♂	174	58.6	4.1	R	0.29	4.3	65	66	1.0	Type II
						E_1	0.84	7.3	110	67	3.9	PI
						E_4	1.39	9.6	172	56	9.8	
						E_{max}	1.70	10.4	184	56	14.4	

[a] Type of anatomy in right ventricle outflow tract prior to operation and residual defects.
[b] Diagnosis established at cardiac catheterization.

Uppsala (4 cases) using patch in the ventricular septal defect (VSD) in all cases. An RV outflow tract patch was used in 10 patients and in one of them it was extended into the main pulmonary artery.

I 9 of the patients postoperative haemodynamic assessment including cardiac catheterization had been performed elsewhere usually within one year after the operation (range 1 month 3 years) whereas the remaining patients unwilling to undergo another cardiac catheterization were investigated only clinically. Five patients were found to have no residual lesion according to the criteria stated under Methods. Another 2 patients had a minimal VSD with no detectable shunt. Thus 7 patients could be regarded as having excellent operative results. Three patients had VSD with small left-to-right shunt. Pulmonary valve stenosis (PS) was present in 5 patients and pulmonary valve incompetence (PI) in 10. The operative results were considered acceptable in all patients. All subjects were in sinus rhythm and none recei ed any medication at the time of the tudy. Individual anthropometric data are listed in Table I as well a detailed presentation of residual defects present.

PROCEDURE

The investigation was carried out St Goran Children Hospital in March–Nov 197 . The patients were hospitalized for 3–4 days during the study. On the second day they performed preliminary test designed to evaluate their aerobic working capacity. The actual exercise study was performed in the morning of the third or fourth day after light meal. Polyethylene catheters 10 cm long, were introduced percutaneously into brachial artery and cubital vein. Simultaneous determinations of cardiac output, oxygen uptake, ventilation and blood lactate concentrations were done at rest in the supine position and during exercise in the sitting position. The exercise was performed on an electrically braked bicycle ergometer (Elema) at pedal rate of 60 rev/min. Two submaximal work loads corresponding to about 50 and 80% of maximal oxygen uptake were performed. Maximal exercise was also done at the highest possible work load the patient could perform for 5–7 min as judged by the information obtained from the preliminary test. The patients were allowed 5–10 min pause between the submaximal exercise and 15 min rest before maximal exercise. This was preceded by 3 min "warming up" period corresponding to about 50% of maximal aerobic power. A blood volume determination preceded the exercise study. N adverse effects were noted in any patient during the study.

COMMENT

The "levelling off" criterion (2) for maximal load on the oxygen transporting system was not strictly used in this study. However in the test preceding the actual study the intention was to establish the ma imal load on which the subject worked to exhaustion in about 6 min. From this maximal load and subjec

Table II *Oxygen uptake ventilation cardiac output stroke volume heart rate haemoglobin and blood lactate concentrations at rest and during exercise in 10 female and 8 male corrected patients with TOF (means ±S D)*

Submax I submax II and maximal load averaged respectively 279 472 and 644 kpm/min for the female and 460 747 and 979 kpm/min for the male subjects

		Rest	Submax I	Submax. II	Max. work
V_{O_2} (l/min STPD)	♂	0.28±0.03	1.18±0.29	1.72±0.28	2.08±0.27
	♀	0.23±0.03	0.84±0.11	1.24±0.20	1.51±0.26
V (l/min BTPS)	♂	8.0±1.1	29.3±6.2	43.9±6.6	69.1±14.0
	♀	7.6±1.9	25.0±3.6	36.9±6.0	56.2±12.0
R	♂	0.76±0.02	0.85±0.04	0.91±0.03	1.04±0.05
	♀	0.81±0.08	0.85±0.02	0.91±0.04	1.01±0.05
$\dot{Q}$ (l/min)	♂	5.3±0.8	9.4±1.4	12.2±1.4	13.2±1.3
	♀	4.5±0.6	7.9±1.7	9.4±1.8	10.8±2.3
HR (beats/min)	♂	67±10	112±16	155±14	179±8
	♀	74±9	113±9	151±10	175±6
SV (ml)	♂	79±12	86±18	80±15	74±9
	♀	61±6	69±13	62±13	62±13
Hb (g/100 ml)	♂	13.8±1.2	14.5±1.6	15.4±1.5	15.9±1.8
	♀	12.8±1.0	13.6±0.9	14.1±0.8	14.5±0.9
Lactate (mmol/l)	♂	0.9±0.3	3.1±0.7	5.8±2.0	11.4±2.6
	♀	0.8±0.3	3.4±1.1	5.8±2.6	10.3±3.2

tive evaluation by the investigator as to whether this load really represented the individual s maximum, the load in the actual test was chosen The lactate values obtained were >9 mmol/l in 14 of the 18 subjects investigated (1) It is therefore concluded at the mean deviation for maximal oxygen uptake m the 'true value is marginal

METHODS

Cardiac output ($\dot{Q}$ l/min) was determined by the dye-dilution technique using indocyanine-green (Cardio-green®) as the indicator substance and a Beckman densitometer as the recording unit. The amount of dye which was injected with a calibrated syringe into the cubital vein varied from 0.80 to 1.73 ml depending on the size of the patients and the work performed, the concentration being 10 mg/ml. Arterial blood was drawn through a cannula inserted in the brachial artery at a constant rate of 55 ml/min. The blood was kept sterile and reinfused after each determination. Duplicate $\dot{Q}$ determinations were made at rest and on each exercise level. Blood for 4-point calibration curves was obtained during maximal exercise in all subjects. The dye-dilution curves were calculated according to the method of Kinsmann et al (29) including manual semilogarithmic plotting, extrapolation and planimetry of the curve areas. The error of the method was calculated from the difference between two consecutive steady state determinations in which the mean difference in heart rate did not exceed 5 beats/min Fifty-two pairs of observations were analysed varying from 3.9 to 15.5 l/min and the error was found to be 8.9%

Heart rate (HR beats/min) was calculated from continuous ECG tracings, counting over a period of 15 sec. HR_{max} is defined as the value obtained at exercise breaking point.

Oxygen uptake (V_{O_2}, l/min STPD) was determined using the Douglas bag technique and the volume of expired air (V l BTPS/min) measured in a calibrated Tissot spirometer Fractions of O_2 and CO_2 in samples of expired air were analysed by the Micro-Scholander method (39).

Arterial *haemoglobin* concentration (Hb g/100 ml) was measured by the cyanmethaemoglobin method and *haematocrit* (Hct) with a high-speed centrifuge

Arterial lactate concentration (lactate, mmol/l) was measured enzymatically (11). *Total blood volume* (l) was measured by the ^{131}I albumin method (43). Values obtained were corrected for the difference between body Hct and central Hct.

Heart volume (HV ml) was calculated according to Jonsell (25).

The presence of *residual heart lesions* was established from previous postoperative catheterization (9 patients) and/or from physical examination Significant residual PS was considered present if a peak systolic gradient of ≥30 mmHg over the RV outflow tract and/or pressure in RV ≥50 mmHg was present at the follow-up catheterization. A PS was also said to be present if a ≥3/6 ejection systolic murmur was detected over the pulmonary valve area. A VSD was considered to be present either if proven at catheterization or if pansystolic murmur (not due to tricuspidal insufficiency) was found. PI was assessed by length and intensity of the diastolic regurgitant murmur

The Wilcoxon rank sum test was used to test the possible influence of a particular residual defect and of the use of a

Table III *Correlation matrix for maximal oxygen uptake and some dimensions and functional capacities of the oxygen transport system in 18 totally corrected adult patients with TOF*
n=no. of observations

	$\dot{V}O_{2\,max}$ (l/min)	$\dot{V}O_{2\,max}$ (l/min/ kg b wt.)	$\dot{Q}_{max}$ (l/min)	SV_{max} (ml)	HR_{max} (beats/min)
BSA (m_2)	.740*** =17	−.058 n.s. n=17	.606* n=18	.677* =18	−.289 n.s. =18
V_{max} (l)	.512* =17	.567* n=17	.403 n.s. =18	.334 n.s. =18	.169 n.s. =18
HV (ml)	.486 n=17	−.019 n.s. =17	.267 n.s. =18	.322 n.s. =18	−.254 n.s. =18
Blood vol (l)	−.038 n.s. n=16	.489 n.s. =16	.274 n.s. =17	−.319 n.s. =17	.260 n.s. =17
$\dot{Q}_{max}$ (l/min)	.846*** =17	.553 =17	–	.974*** =18	.057 n.s. =18
SV_{max} (*ml*)	0.816 ** =17	.422 n.s. =17	0.974 ** =18	–	−.167 n.s. =18
HR_{max} (beats/min)	.155 n.s. =17	.517* =17	.057 n.s. =18	−.167 n.s. =18	
$\dot{Q}_{max}$×1.34×Hb	.841 ** =17	.503 =17	.866*** =18	.834 ** =18	0.87 n.s. =18

right ventricular outflow patch on stroke volume and $\dot{V}O_{2\,max}$. Otherwise current statistical methods were used (41). The degree of probability was designated as follows. $0.05<p$ not significant (n.s.); $0.05\geq p>0.01$ probably significant ( ), $0.01\geq p>0.001$ significant (*) $0.001\geq p$ highly significant (**).

RESULTS

Individual and mean values are given in Tables I and II and a correlation matrix in Table III.

The mean maximal *oxygen uptake* ($\dot{V}O_{2\,max}$) was 2.08 l/min for the males and 1.51 l/min for the females. This corresponds to a value of 30.5 ml/min/kg and 25.5 ml/min/kg for the males and females, respectively when related to body weight and to values of 1.13 l/min/m² 0.92 l/min/m² when related to BSA. Both the absolute and relative values for $\dot{V}O_{2\,max}$ are lower than normal for Swedish males and females of corresponding ages (1, 2). Individual values were all below −2 S.D. The correlation between $\dot{V}O_{2\,max}$, on the one hand and $\dot{Q}_{max}$ and SV_{max} on the other was highly significant. No correlation was found with blood volume or HR_{max}.

Mechanical efficiency

$$\left(\frac{\text{mechanical work performed (kpm/min)}\times 100}{427 \cdot 4.9\ (\dot{V}O_{2\,work}-\dot{V}O_{2\,rest})}\right)$$

was found to be normal in both the male and the female group (1) the mean being 22.9±1.4% at submaximal load II.

Cardiac output was low when related to $\dot{V}O_2$ both during submaximal and maximal work (Fig. 1). Half of the observations were below −2 S.D. of the regression line for young males found by Ekblom et al (12) using techniques similar to those in the

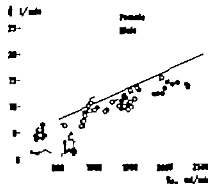


Fig. 1. Cardiac output in relation to oxygen uptake at rest in the supine position and during exercise in the sitting position in 18 totally corrected adult patients with TOF. The regression line ($y=5.72x+5.13$ S.D. ±1.44) is calculated from data of Ekblom et al. (12). The present regression equation: $y=4.56x+3.8$ S.D. ±1.39. Significance of difference between lines $0.05\geq p>0.01$

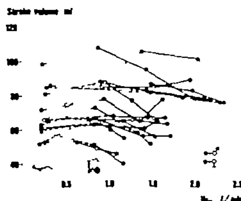


Fig 2 Individual values for stroke volume in relation to oxygen uptake at rest in the supine position and during exercise in the sitting position in 18 totally corrected adult patients with TOF

present investigation Only one observation was above this regression line $\dot{Q}_{max}$ was 13 2 l/min for the males and 10 8 l/min for the females corresponding to cardiac indices of 7.2 and 6 6 l/min/m² for males and females, respectively No correlation was found between $\dot{Q}_{max}$ and HV blood volume or HR_{max}.

Stroke volumes (SV ml) at rest in the supine position were small when compared with normal volumes (3 6 28) The mean value for males was 79 ml (range 66–99) and for females 61 ml (range 5?–72) Corresponding values for stroke index were 43 4 and 6 respectively SVs at submaximal exercise I re slightly but significantly higher (0 01≥p>0 001) han at rest in the supine position During exercise there was a fall in SV with increasing work loads mean SV being 76 ml during submaximal exercise I 70 ml during submaximal exercise II and 67 ml during maximal exercise (Fig ?) The difference between SV during exercise I and maximal exercise was highly significant (0 001≥p). In 8 patients the fall in SV was >10 ml. The fall in SV and the size of SV_{max} could not be correlated to any particular residual lesion. No correlation existed between SV_{max} and blood volume HV or HR_{max}

Mean maximal *heart ate* was 184±9 beats/min for the male group and 179±7 beats/min for the female HR related to relative work is illustrated in Fig 3

Total blood volume was 4 78±1 10 1 or 71±15 ml/kg b.wt. in the male group and 4.20±0 43 1 or 73±13 ml/kg b wt. in the female.

Heart volumes were larger than normal. In the male group a mean value of 864±150 ml was found corresponding to 465±36 ml/m² when related to BSA In the female subjects the values were 714±136 ml and 439±85 ml/m² respectively

Values for Hb and lactate concentrations as well as ventilation are summarized in Table III A detailed statement of spirometric data pulmonary ventilation, gas exchange and acid–base balance has been given in an earlier publication (7)

DISCUSSION

The values obtained for maximal oxygen uptake are low for both sexes They are almost identical with the figures found in totally corrected TOF patients by Epstein et al in a recently published investigation (14) and represent a reduction of 30–40% compared with normal figures (1 ?) These findings show that in spite of a successful anatomic repair no true normalization of the aerobic work capacity occurs However in a study from this department of 16 only shunt-operated TOF patients of comparable age the $V_{O_2 max}$ was found to be only 18 0 ml/kg/min Thus about 60% higher values were found in the totally corrected subjects This corresponded well with the subjective evaluation made by the patients themselves who felt that their exercise tolerance had increased considerably after intracardiac repair

Values for cardiac output related to oxygen uptake were lower than in normal subjects (Fig. 1) This finding is contrary to earlier reports (9 17 18 33 40)

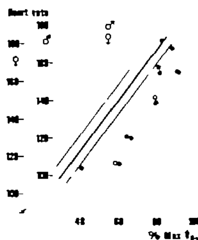


Fig 3 Individual values for heart rate (beats/min) during submaximal work in the sitting position in relation to oxygen uptake expressed percentage of the maximal oxygen uptak in 18 totally corrected adult patients with TOF Regression line with S.D calculated from data of Åstrand (2).

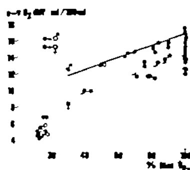


Fig 4 Arteriovenous oxygen difference in relation to oxygen uptake expressed as percentage of maximal oxygen uptake i 18 totally corrected adult patients with TOF The regression lines, females $y=0.078x+6.40$ and males $y=0.072x+9.61$ are calculated from data of Åstrand et al. (3). The present regression equations, males $y=0.087x+7.11$ and females $y=0.067x+7.36$, were not significantly different from those of Åstrand.

which state that a normal increase in $\dot{Q}$ occurred with exercise. In the latter studies however haemodynamic evaluations were performed with the patients working at low exercise loads. The finding of an altered relationship between $\dot{V}_{O_2}$ and $\dot{Q}$ is however in agreement with Epstein s results from 10 corrected patients with TOF without significant residual lesion. The same situation has been found to exist in other types of congenital heart disease e g atrial septal defect pulmonary valvular stenosis mitral stenosis aortic insufficiency (4 13 14 22, 26, 37) both before and after total correction In the present series no correlation was found between the decrease in $\dot{Q}$ relative to $\dot{V}O_2$ and the type of anatomy in the RV outflow tract, the use of outflow patch or the presence of a particular residual lesion. It is thus likely that the "inadequacy" of the $\dot{Q}$ response to exercise is less related to the type of congenital heart malformation than to the myocardial function itself.

The low $\dot{Q}$ relative to $\dot{V}_{O_2}$ led to a higher-than-normal AV oxygen difference This does not reflect a more effective oxygen extraction and/or a changed distribution of the left ventricular (LV) output e.g from visceral organs to working muscles, since the AV oxygen difference was found to be normal when relative work was used as frame of efer ence (Fig. 4) Furthermore the AV oxygen dif ference during maximal exercise expressed as percentage of oxygen binding capacity of the blood was found to be normal (73 %)

An analysis of the subfunctions of the cardiac output, i.e heart rate and stroke volume reveals that the main cause of the low $\dot{Q}$ was the small SVs found 74 ml in the male and 6_ ml in the female group during maximal exercise in the sitting position. A highly significant correlation existed between SV_{max} and $\dot{V}_{O_2\,max}$ The reason for the small SVs found cannot be fully elucidated from the results of this study However some conclusions could be drawn from the findings in the postoperative catheterizations performed on some of our patients and from previous investigations

The SV depends not only on the anatomical size of the ventricles but also on the venous return to the heart, the resistance to emptying of the entricles and the contractibility characteristics of the heart muscle. Some controversy exists concerning the size of the LV in TOF Some investigators have found the LV to be normal or slightly small (31 34) and an underdevelopment of this chambe severe enough to be of haemodynamic significance is said to be present in less than 1% of the patients (36) However Jamarkard et al (23) convincingly showed that cyanotic patients with TOF more than 2 years of age had smaller left HV than normal With suc cessful shunt operation or total correction an increase in LV end-diastolic volume as well as LV mass occurred to values significantly higher than normal In spite of this the LV ejection fraction remained subnormal This latter finding was attributed to a depressed LV function and consequently not to small left HV

It is well documented that stroke volume is influenced by the degree of physical activity and that it can be increased by physical training (12 15 28 38) Our patients were definitely more inactive than nor mal during their childhood and they are probably less active physically also after total correction In the present study only patients stated that they regularly participated in strenuous physical activities. The vast majority of our patients have an occupation which could classify them as white-collar workers The reduction in SV might therefore theoretically be explained by small circulatory dimensions sec ondary to prolonged physical inactivity It has been shown that a high degree of interdependence exists between different components of the oxygen transport system and the degree of physical activity or $\dot{V}_{O_2\,max}$ (20). The correlation matrix (Table III) shows that this was the case in our subjects too with one exception namely the dimensions of the cardiovascular system. Though the SV was corre



Fig 5 Individual values for stroke volume during maximal exercise in relation to end-diastolic pressure in the right ventricle at rest in 9 subjects with totally corrected TOF

lated to $V_{O_2 max}$ this was not the case with blood volume or heart volume Neither was there any intercorrelation between stroke volume blood volume and heart size If the reduction in SV were to be explained by low physical activity alone one would have expected such correlations to exist. However it cannot be excluded that physical inactivity was a contributory cause of the reduction of the SVs It would therefore have been of interest to see whether our patients SV would have increased after organized physical training

One explanation of the small SVs may be an ormal contractile response of the ventricles and tered compliance characteristics of the ventricular myocardium (14 23) Several factors such as the myocardial changes in the right ventricle secondary to the long-standing muscular hypothropy non-contractile parts of the myocardium corresponding to the ventriculotomy scar the patch in the ventricular septum and the RV outflow tract have to be considered as well as the RBBB that is usually present.

An RV outflow tract gradient is usually present even in successfully operated TOF patients (9 14 30 40) This gradient increases during exercise (14 40). To maintain an adequate SV in this situation there are three possibilities to increase the RV systolic pressure to prolong the ejection time and to increase the diastolic filling (22) Each of these compensatory mechanisms has been found in TOF (9 14 23 40). However SV decreased during exercise in the present group If the RV is the limiting factor for maintenance of the SV this indicates that the above mentioned mechanisms are not sufficient. At higher HR a longer duration of systole results in a shorter diastole This may hamper ventricular filling when an increased filling pressure is needed and the ejection time is prolonged One may speculate whether this causes the fall in SV during exercise in some of our patients Support for this theory may be the observed negative correlation between the end-diastolic pressure in RV and the size of SV_{max} ($0.01 \geq p > 0.001$) and SV/BSA ($0.05 \geq p > 0.01$) found in our series (*Fig. 5*) However this latter finding must be viewed with caution due to the small number of observations and the fact that data of end-diastolic pressure and SV were obtained on different occasions

The slightly low maximal heart rate agrees with findings of Hurvitz and Goldberg (21) that the HR_{max} was lower than normal not only before, but also after operative correction of different cardiac lesions. Similar or related observations have been reported by others (16 26, 27 42) The reason for the subnormal HR_{max} in cardiac patients is not fully understood One possible explanation is that the subjects stop exercising for other reasons than circulatory such as e g. dyspnoea or hypoxaemia (26). This might be the explanation in uncorrected but hardly in totally corrected TOF patients since their ventilatory response to exercise is normal and since they do not have hypoxaemia (7). Another explanation may be that the lower maximal cardiac frequency represents a physiological adaptation to the haemodynamic situation A lower-than-normal heart rate may be needed to allow optimal diastolic filling If this explanation is valid one would expect not only the HR_{max} to be lower than normal but also the HR at submaximal loads That this is the case is shown in Fig. 3 Other possible explanations may be related to muscular ventricular hypertrophy or to the presence of conduction disturbances e.g. RBBB It should however be noted that in the series of Hurvitz and Goldberg (21) patients with conduction disturbances were excluded.

In the present study the decrease in aerobic capacity could not be correlated to the anatomy of the RV outflow tract or to the use of an outflow patch Though the number of patients studied was small, this tends to substantiate the findings of others (9 18, 24 33) that TOF patients tolerate PI reasonably well. This seems to be the case also when correction is undertaken at adult age

There was no complete normalization of physical

work capacity in our patients the majority of whom were corrected at adult age Future studies will show whether a better normalization is achieved if total correction is undertaken in early life. This might perhaps be expected if the TOF patients perform a normal degree of physical activity during the years when growth of circulatory dimensions takes place The reduction in $V_{O_{2max}}$ was moderate and did not interfere with everyday activities. However it means that at a certain degree of activity our subjects utilized a greater portion of their aerobic work capacity A greater portion of the cardiac reserve was mobilized. This may prove to be a disadvantage as the patients grow older and are subjected to the ageing processes in the heart and to ischaemic heart disease.

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OXYGEN UPTAKE ARTERIAL BLOOD GASES AND BLOOD LACTATE CONCENTRATION DURING SUBMAXIMAL AND MAXIMAL EXERCISE IN ADULT SUBJECTS WITH SHUNT-OPERATED TETRALOGY OF FALLOT

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Abstract. Ten female and six male adult subjects with shunt-operated tetralogy of Fallot have been studied at rest and during submaximal and maximal exercise on an average 20 years after the palliative operation. There was considerable reduction in the aerobic work capacity maximal oxygen uptake (V_{O_2}) being 1 00 l/min STPD Though entilation (V_E) was out of proportion to V_{O_2} as indicated by an abnormally high ventilation equivalent (52.1), values for $V_{E_{max}}$ were low (50.8 l/min BTPS) and of approximately the same order as in comparable group of totally corrected TOF patients In spite of increased V_E in relation to V_{O_2} the P CO_2 increased from 30 mmHg at rest to 49 mmHg during maximal exercise, while P O_2 decreased from 60 mmHg at rest to 44 mmHg during submaximal exercise I. During heavier exercise no further fall was noted. Base excess decreased from −2.9 to −9.8 mEq/l Thus combined respiratory and metabolic acidosis was at hand during exercise, the metabolic component, however being normal Maximal blood lactate concentrations were low (5.8 mmol/l) and contrasted with the high intramuscular lactate concentrations earlier reported in some of the patients. The low values found for $VO_{2\ max}$ indicate that palliative operative procedure in TOF is no alternative to an intracardiac repair in the long-term course. The two main factors limiting exercise tolerance were acidosis and accumulation of lactate within the muscle cell

Tetralogy of Fallot (TOF) is characterized by an obstruction of the outflow tract of the right entricle in combination with a non-pressure limiting ventricular septal defect, enabling shunting of the blood from either the left ventricl to the right or vice versa. Before the time when palliative shunt operations were introduced the prognosis for TOF was fairly bad (24) The determining factor for the prognosis in the individual subject was the degree of reduction of the blood flow to the lungs Therefore the aim of the first operations in TOF was to increase the pulmonary blood flow (7). These operations though only palliative improved the prognosis (21)

Reports on the long-term prognosis in palliated TOF are now available (8, 15 23 29) However data concerning the aerobic working capacity and reaction to exercise are few in adult subjects with TOF Still an anticipated reduced working capacity is used as an argument in fa our of total correction. The strength of this particular argument, however depends on how severe the reduction is and how handicapped palliated patients are in their activities of daily life.

Thus the aim of this study was to examine subjects with palliated TOF who had lived for rather a long time after their shunt operation, in respect of the following questions

1 The adaptation of ventilation, oxygen uptake and gas exchange during submaximal and maximal exercise

2. The effect of hypoxaemia during exercise on the anaerobic metabolism as judged from the oxygen deficit and the blood lactate concentration.

SUBJECTS

Six male and ten female subjects, aged 22–35 years, were studied. They all belonged to the first shunt-operated group of TOF patient in Sweden. Data from follow-up 5.5 years after the shunt-operations have earlier been published by Möller (21) Of the 30 subjects who had not been subjected to an intracardiac repair 1 were still alive from whom 16 *subjects were recruited all having Blalock Taussig* anastomoses As the general opinion is and has for several years been that patient with TOF should hav an intracardiac repair performed around the age of 5 years (20), and as 20 years had elapsed since the original shunt opera-

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Table II. *Mean values ± S.D. for ventilation, oxygen uptake, oxygen deficit and heart rate during submaximal and maximal exercise in shunt-operated TOF*

No. of subjects given within parentheses

	Submax. I (221 kpm/min)	Submax. II (373 kpm/min)	Max. exercise (516 kpm/min)
$\dot{V}_E$ (l/min BTPS)	23.7±8.3 (14)	36.7±8.9 (13)	50.8±18.6 (16)
$\dot{V}_{O_2}$ (l/min STPD)	0.65±0.15 (14)	0.90±0.19 (13)	1.00±0.30 (16)
$\dot{V}_E/\dot{V}_{O_2}$	36.6±10.5 (14)	42.5±12.8 (13)	52.1±17.5 (16)
R	0.80±0.08 (14)	0.92±0.08 (13)	1.00±0.09 (16)
Oxygen deficit (l)	0.60±0.33 (13)	1.16±0.43 (13)	1.83±0.56 (16)
HR (beats/min)	118±16 (14)	143±19 (13)	167±13 (16)

Clark type microelectrode with polypropylene membrane, P_aCO_2 and base excess were obtained from a curve nomogram, the values being corrected for low arterial saturation when present (27). The reproducibility of the methods in our laboratory has been reported elsewhere (13). Hb concentration was measured spectrophotometrically (16) and lactate concentration enzymatically (10).

Calculations

Oxygen deficit was calculated assuming a normal mechanical efficiency of 22% (1, 12). The difference between the $\dot{V}_{O_2}$ calculated from the assumed mechanical efficiency and the total measured $\dot{V}_{O_2}$ during the whole work period of 4 min was taken as the oxygen deficit at that particular load. In the two subjects whose work time was 6 min, the first portion of expired air was collected during the first 3 min and the second portion during the last 2 min of exercise. Oxygen deficit in these two subjects was calculated according to Karlsson (19).

Comments

Four-minute exercise periods were chosen, as it has been shown that during work of longer duration an i.m. utilization of lactate takes place in normal subjects (18). Thus blood lactate concentration in longer exercise periods does not fully reflect the anaerobic metabolism. However, short work periods mean that values for $\dot{V}_{O_2}$ during submaximal exercise are not quite comparable with those for longer exercise periods. This was one factor which made calculations of mechanical efficiency in our subjects impossible.

Table III. *Mean values ± S.D. for arterial blood gases, haemoglobin and blood lactate concentrations at rest and during exercise in shunt-operated TOF*

No. of subjects given within parentheses

	Rest	Submax. I (221 kpm/min)	Submax. II (373 kpm/min)	Max. exercise (516 kpm/min)
P_aO_2 (mmHg)	60±8.0 (13)	44±8.6 (10)	45±5.0 (9)	42±7.1 (13)
P_aCO_2 (mmHg)	30±4.0 (13)	42±9.9 (10)	41±7.8 (9)	49±13.8 (13)
pH	7.43±0.02 (13)	7.32±0.06 (10)	7.30±0.06 (9)	7.22±0.06 (13)
Base excess (mEq/l)	−2.9±1.9 (13)	−5.1±1.5 (10)	−6.9±2.5 (9)	−9.8±2.5 (13)
Hb concentration (g/100 ml)	18.2±4.7 (16)	19.0±5.0 (7)	18.8±5.0 (8)	18.6±5.2 (11)
Blood lactate concentration (mmol/l)	0.8±0.3 (16)	2.2±0.6 (11)	3.3±0.9 (11)	5.8±1.5 (15)

RESULTS

The ten female and six male subjects worked at two submaximal loads beside the maximal one. The latter averaged 450 kpm/min for the female 625 kpm/min for the male subjects and 516 kpm/min for the total group The work times at maximal exercise loads were 3.9 3 7 and 3 8 min respectively Mean values of the data obtained during exercise are given in Tables II and III Oxygen uptake was 0.65 l/min and 0 90 l/min at submaximal load I (221 kpm/min) and submaximal load II (373 kpm/min) respectively During maximal exercise a value of 1 00 l/min was obtained Total ventilation increased from 23 7 l/min during submaximal 1 to 50.8 l/min during maximal exercise (Fig 1) giving a ventilation equivalent of 52 1 during maximal exercise

Oxygen deficit was 0.60 l during submaximal I 1 16 l during submaximal II and 1 83 l during maximal exercise Heart rate increased from a resting value of 77 to 167 beats/min during maximal exercise P O_2 was 60 mmHg at rest. During exercise it decreased to 44 mmHg at the lowest work load but no further fall occurred at higher work loads P CO_2 was 30 mmHg at rest and increased during exercise to 42, 41 and 49 mmHg (Fig 2) The increase between submaximal I and maximal exercise is statistically significant ($p < 0.05$) Base excess dropped from −2.9 to −9 8 mEq/l during maximal exercise and erial pH from 7 43 to 7.22 (Fig 2) Arterial blood te concentration during maximal exercise was 5 8 mmol/l Arterial Hb concentration was found 19 g/100 ml both at rest and during exercise

COMMENTS

In this study maximal oxygen uptake was defined as the value for oxygen uptake found at the heaviest load on which the subjects could work for 4 min according to information obtained at the preliminary test. The very high values for V_E/V_{O_2} and R support the subjective impression of the investigator that the patients worked at or near their maximum Thus the obtained values for V_{O_2} at maximal work loads could be looked upon as acceptable for $V_{O_2\,max}$

DISCUSSION

The few reports published on aerobic working capacity in subjects with TOF have shown low values (11 12 14 28 30) However the reported materials differ from ours In Taylor s material (30) there were only 9 of 16 reported subjects with non-

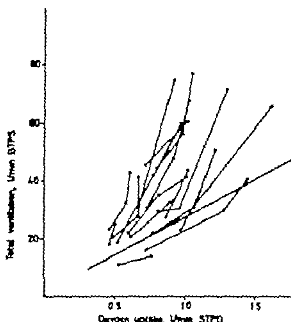


Fig 1 Individual values for total ventilation in relation to oxygen uptake at submaximal and maximal exercise loads in subjects with shunt-operated TOF Solid line denotes regression line for normal subjects (4).

totally corrected TOF and most of them were acyanotic In the material of Gold et al (14) all 17 subjects studied had palliated TOF but in most of them the shunt was a Potts anastomosis. Also the material of Davies and Gazetopoulos (12) differs from ours Of their 20 subjects 13 had TOF but only 5 of them had had a shunt operation performed In the material of Crawford et al (11) the 21 TOF subjects were all palliated 13 with a Blalock Taussig anastomosis and the rest with Potts anastomosis (22) the latter however having an increased pulmonary blood flow Thus our results are not quite comparable with those reported by others On the other hand our series has the longest follow-up and is uniform as in all cases the anastomosis performed was of the Blalock Taussig type (7). Therefore our values for working capacity add information about the situation in adult shunt-operated subjects with TOF

$V_{O_2\,max}$ was around 30% of the normal in Sweden (1) The six male subjects had slightly higher values 1 21 l/min and 22.2 ml/kg/min than the female subjects 0 88 l/min and 15.3 ml/kg/min in spite of a deeper cyanosis and higher Hb concentration (Table I) Even if the difference in body composition is considered (2) higher values for the aerobic power were found in the male subjects The reason for this

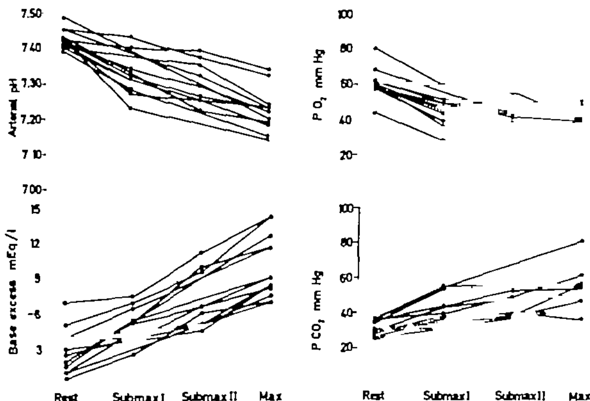


Fig. 2. Individual values for arterial oxygen tension (P O_2), arterial carbon dioxide tension (P CO_2), arterial pH and base excess at rest, at submaximal and maximal exercise load in subjects with shunt-operated TOF.

is obscure. However, all six male subjects were physically active in their daily work, while many of the females were relatively inactive. Thus the degree of cyanosis as reflected in the arterial saturation at rest and in the Hb concentration do not always give information about the degree of lowered working capacity.

Many activities of daily life require a V_{O_2} around or above 1 l/min (2). This means that shunt-operated adult subjects with TOF are easily forced to a working level near the maximal. If therefore aerobic power is used as a criterion of these subjects' situation, it speaks strongly in favour of an intracardiac repair. However, many of the subjects stated that they experienced no or only mild discomfort during daily life. The same observation was made by Gold et al. (14). This emphasizes that subjective information about exercise tolerance from a subject with TOF must be looked upon with a certain degree of caution.

In another study (4) the reduced values for vital capacity and total lung capacity in these subjects as well as in a comparable group of totally corrected TOF patients were reported. It was also found that the totally corrected patients had a normal ventilatory response to exercise. Though $V_{E_{max}}$ was low (61.9 l/min) it matched the $V_{O_2\,max}$, giving a normal V_E/V_{O_2} ratio and did not constitute a limiting factor for working capacity. In the present study a slightly lower value for $V_{E_{max}}$ was obtained (50.8 l/min) in spite of the roughly 40% lower $V_{O_2\,max}$. This resulted in a very high V_E/V_{O_2} ratio during maximal exercise. This should not be taken as an absolute indication, however, that excessive V_E or increased respiratory work limits the working capacity in palliated TOF patients, as their $V_{E_{max}}$ is, if anything, lower than in the comparable group of totally corrected subjects.

An abnormal V_E/V_{O_2} ratio was present also during submaximal exercise (Fig. 1) and possibly also at rest as judged by the low P CO_2. The hyperventilation present at rest cannot be fully explained by the experimental situation (1) as the P CO_2 was lower than in totally corrected TOF subjects in the same situation (4). The subjects hyperventilated in spite of

normal pH and hypocapnia, leaving hypoxia as a possible stimulus to ventilation at rest.

It is obvious that part of the ventilations is "wasted" in cyanotic patients with TOF as blood is shunted past the lungs at cardiac level. An increase in dead space ventilation within the lung in palliated TOF patients has been reported (14 28) and should if present, contribute to the wasted ventilation. The finding in the present series of a P_aCO_2 of 30 mmHg at rest must imply in the presence of an intracardiac right-to-left shunt, a still lower PCO_2 in the pulmonary veins indicating an alveolar hyperventilation. Whether the increased total ventilation leads to a uniform proportional increase in dead space ventilation and alveolar ventilation during exercise is not known. The main component of the wasted ventilation however is most likely secondary to the increasing right-to-left intracardiac shunt during exercise and not to an increase in the physiological dead space within the lung.

When changing from rest in the supine position to exercise in the sitting position an increase in P_aCO_2 was found. Even slightly higher values were obtained during maximal exercise. The possibility that the altered body position in itself may partly explain the results cannot be excluded. However shunting at the cardiac level of venous blood with an increased CO_2 tension during exercise seems to be the main reason for the values found. The subjects were unable to compensate for the normal metabolic acidosis occurring during exercise with a respiratory alkalosis as a respiratory acidosis existed in spite of high $\dot{V}_E/\dot{V}O_2$ ratio.

A fall in P_aO_2 occurred during exercise due to an increasing intracardiac right-to-left shunt (20). However during heavier exercise no further fall in P_aO_2 was noted. This may partly be explained by an increasing pulmonary blood flow due to left-to-right shunts through aortopulmonary collateral circulation and via the Blalock Taussig anastomosis. The flow through these anastomoses increases during heavy exercise since the mean pressure in the aorta increases while the pressure in the pulmonary arteries remains unchanged. However in the presence of an increasing P_aCO_2 a corresponding decrease in S_aO_2 must take place. The unchanged P_aO_2 must therefore partly be attributed to a right displacement of the oxyhaemoglobin dissociation curve secondary to increasing acidosis.

Judged by the base excess value obtained during maximal exercise the metabolic component of the acidosis was not abnormally pronounced. However due to the inability to eliminate CO_2 a respiratory component was added to the acidosis resulting in subnormal pH values.

Blood lactate concentration was normal at rest. During exercise an increase was found but values during maximal exercise were considerably lower than normal. A subnormal blood lactate response to exercise in patients with cyanotic heart diseases has been found by others (28). This has formed the basis for the statement that these patients stop working for other reasons than muscular or that no hypoxaemia incapacitated the muscle (28). In the present series the normal metabolic component of the acidosis, subnormal blood lactate values and the finding of similar P_aO_2 values during exercise of different intensities tend to substantiate this statement. However in the exercising muscle in those of our patients investigated the lactate concentration was found to be high (6). This was attributed to circulatory factors and inability to eliminate lactate from the muscle cell. This means that the blood lactate concentration does not fully reflect the anaerobic metabolism during exercise in subjects with TOF.

Heart rate at maximal exercise was 167 beats/min. This "maximal heart rate" is almost 30 beats lower than what is considered normal (1 2). Whether or not this is due to a real lower maximal heart rate is difficult to say. It is possible that the subjects reach the exercise breaking point before the maximal heart rate potential is reached. On the other hand studies on comparable subjects with TOF who had had an intracardiac repair show that they attain only a slightly higher maximal heart rate (5).

From the above discussion it is concluded that the two main factors limiting exercise tolerance were acidosis and accumulation of lactate within the muscle cell. Both are related to circulatory factors the acidosis being due to CO_2 accumulation secondary to low pulmonary blood flow and the lactate accumulation possibly to altered blood flow to the muscle.

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HAEMODYNAMIC EFFECT OF ATRIAL TRIGGERED VERSUS FIXED RATE PACING AT REST AND DURING EXERCISE IN COMPLETE HEART BLOCK

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Abstract The central haemodynamics at rest and during exercise have been studied in 25 patients with complete AV block who were treated with fixed rate (FRP) and atrial triggered pacemakers (ATP). The aim of the investigation has been to study the effect of synchronized atrial contraction for the filling of the ventricles and for the cardiac output ($\dot{Q}$). Pressures and $\dot{Q}$ have been determined during heart catheterization. The P wave for triggering the atrial synchronized pacemaker has been obtained with an electrode in close contact with the atria, introduced by means of mediastinoscopy The study consists of two series. In the first series (12 patients) the central haemodynamics were recorded with each patient connected first the FRP (about 70 *impulses/min) and later to the* ATP Most patients were studied both at rest and during exercise, the work loads being identical with both types of pacemakers. $\dot{Q}$ at rest is 10% higher with ATP ($p<0.02$) than with FRP and during exercise 20% higher with ATP ($p<0.01$). Stroke volume (SV) at rest is equal with both types of pacemakers, but significantly larger with FRP during work ($p<0.001$). The left ventricular (LV) filling pressure is significantly lower ($p<0.01$) with ATP at rest, but not during exercise ($p<0.8$). In the second series 13 patients were studied at rest and during exercise. The recordings of pressures and $\dot{Q}$ were first performed with the patient on ATP After 30 min rest an identical study was performed with the patient connected to FRP the rate of which was matched (FRPm) to that previously recorded with ATP $\dot{Q}$ at rest is 18% higher ($p<0.01$) with ATP than with FRPm and during work 8% higher ($p<0.05$) with ATP SV at rest is significantly larger ($p<0.01$) with ATP than with FRPm, whereas during exercise no significant difference is observed between the two types of pacemakers. LV filling pressure at rest is significantly lower on the 5% level with ATP during exercise no significant difference i observed. The investigation shows that in many patients, especially younger ones, treatment with ATP makes it possible to obtain larger $\dot{Q}$ during exercise, and thus to increase the oxygen transporting capacity of the circulation. This beneficial effect may be more pronounced in patients with low compliance of the ventricular myocardium.

In two earlier studies from this laboratory the effect of different ventricular rates on cardiac output and central pressure at rest and during exercise in patients with artificial pacemakers giving impulses at a fixed rate to the right ventricle has been reported (1 3)

Atrial triggered ventricular pacing is now a well established technique for treatment of patients with AV block. There are however rather few studies of the haemodynamic effect of atrial triggered (ATP) versus fixed rate pacing (FRP) and most of them deal only with patients studied at rest (6 18 19) Center et al (9) determined the cardiac output ($\dot{Q}$) at ventricular FRP before implanting an ATP and with ATP 3–12 months after the operation. They demonstrated an increase in $\dot{Q}$ during atrial synchronous pacing both at rest and during exercise. Sowton (22) showed by comparing the haemodynamic effect of ventricular FRP to that of sinus rhythm that the connection of the pacemaker induced an immediate drop in $\dot{Q}$ following the loss of atrial delivery In these studies however increases in stroke volume (SV) returned the $\dot{Q}$ to its original level during the next few minutes In a preliminary study from this department the effect of atrial synchronization during work has been reported in two patients (15) This study indicated a beneficial haemodynamic effect of atrial synchronous pacing.

The present investigation deals with the haemodynamic effect of ATP versus ventricular FRP in a larger material. In one series 12 patients were investigated by cardiac catheterization, at rest and during work, using both FRP at about 70 beats/min and ATP The same procedure was followed with both types of pacemakers The main object was

Table I *Some anthropometric data*

Case no.	Sex	Age (y)	Height (cm)	Weight (kg)	BSA (m²)	HV (ml)	THb (g)
Series I							
1	♂	65	176	74	1.91	960	489
2	♂	65	170	68	1.80	1 100	544
3	♂	60	177	79	1.97	600	664
4	♂	66	175	91	2.06		761
5	♀	50	157	69	1.68	680	520
6	♂	56	175	64	1.80	1 160	493
7	♀	31	167	56	1.63	880	461
8	♀	67	151	64	1.58	490*	353
9	♂	51	170	73	1.84	1 320	623
10	♂	60	183	70	1.93	1 070	766
11	♀	49	163	76	1.81	900	569
12	♂	48	184	79	2.04	1 130	885
Series II							
1	♂	67	174	63	1.78	910	543
2	♀	66	164	76	1.82	830*	
3	♂	61	178	83	2.00	1 180	678
4	♀	30	158	55	1.55	755	349
5	♀	60	156	63	1.61	730*	366
6	♂	54	174	70	1.85	1 070*	
7	♂	64	163	75	1.78	1 300*	
8	♂	53	173	69	1.84	780*	
9	♀	45	161	63	1.65	770	362
10	♀	37	175	77	1.92	890	831
11	♂	70	182	87	2.10	1 080*	868
12	♂	65	179	86	2.06	860*	
13	♂	40	172	59	1.70	1 050	583

Examination made with patient in standing position.

study the circulatory dynamics during spontaneously increasing heart rate with synchronized atrial systole versus a fixed ventricular rate during work. In a second series of 13 patients the FRP was matched to the same rate as that previously obtained under identical conditions with an ATP in the same patient at rest and during work. This study demonstrates the effect of the atrial systole on the central haemodynamics.

MATERIAL

Series I

Twelve patients (4 women, 8 men), aged 31–67 years (mean 56) with complete AV block were studied. Each patient had a moderately enlarged heart (Table I).

The duration of symptoms before electrode implantation and the duration of pacemaker treatment before the study as well as other relevant diseases are presented in Table II. It is evident that the duration of pacemaker treatment before the study is rather short in most patients but in no case shorter than 15 days.

A history of rheumatic affection is suspected in three patients (nos. 3, 6, 12) of whom two have aortic stenosis. In one patient (no. 2) the first spell of AV block III was recorded when he underwent treatment for a myocardial infarction. No definite aetiology could be established in the other seven patients.

Series II

Thirteen patients (5 women, 8 men), aged 30–70 years (mean 55) with complete AV block were studied. Each patient had an enlarged heart, but more markedly enlarged in only one (no. 7) who also had an aortic valvular lesion (Table I).

Table II shows that the duration of pacemaker treatment before the investigation is rather short in all patients but shorter than 15 days in only one patient (no. 4). It appears from Table II that the aetiology of the AV block could be myocarditis in six patients (nos. 1, 3, 5, 6, 7, 9). Two of them (nos. 6, 7) have an aortic valvular lesion and one (no. 9) also has cardiomyopathy. Familial cardiomyopathy is present in three patients (nos. 4, 9, 13). In patients 2, 8, 10, 11 and 12 no definite aetiology could be established although one (no. 10) has hypertension and had a pulmonary tuberculosis at the age of 24.

The patients in series I and II are rather similar regarding age distribution and duration of pacemaker treatment. The mean duration of AV block before initiation of pacemaker treatment is longer in series I than in series II, a fact that should not interfere with the results. The patients in both

Table II *Duration of symptoms before electrod insertion and duration of pacemake tre tment before the study*

Case no.	Duration of symptoms before insertion of pacemaker electrode	Duration of pace maker treatment be fore catheterization	Other relevant diseases
Series I			
1	2 mo.	1 mo.	
2	5 mo.	8 mo.	Infaction 1 y ago short period of AV III
3	5 y	1 mo	Rheumatic fever in 1930
4	6 y	15 d	
5	4 y	15 d.	
6	6 y	6 mo.	Aortic stenosis. In 1957 myocarditis
7	1 y	1 mo.	Air embolism 5 y ago
8	1 mo.	15 d.	Hypertonia
9	2 mo	2 mo.	Cardiac enlargement since 1 y
10	3 mo.	15 d.	
11	4 y	1 mo.	
12	10 y	1 y	Aortic stenosis
Series II			
1	5 mo.	15 d	Myocarditis 20 y ago
2	6 mo.	1 mo.	
3	6 mo.	15 d	Before onset of symptoms pleurisy and pneumonia
4	4 d.	6 d.	Hypertrophic cardiomyopathy
5	1 mo	15 d.	Diphtheria as child. Symptoms started after air-way infect. Myocarditis?
6	6 y	15 d	Rheumatic fever at the age of 35. Slight aortic incomp.
7	1.5 y	1 mo	Combined aortic lesion
8	20 d.	2 mo.	
9	2 y	1 mo.	In 1930 and 1936 rheumatic fever Cardiomyopathy
10	1 y	5 mo.	Pulmonary tbc at the age of 24 Hypertonia
11	15 d	4 mo.	
12	4 mo.	1 mo.	
13	6 mo	3 mo	Cardiomyopathy

series belong to selected group suitable for ATP Atrial fibrillation had never been observed in any of them. Some of the patients, when connected to the FRP temporarily showed interference of sinus rhythm. None had history of coronary insufficiency

After being fully informed all patients consented to participate in the investigations.

METHODS AND PROCEDURE

In all patients transvenous monopolar electrode (Siemens-Elema, EMT 588) (14) had previously been introduced through the right external jugular vein, with its tip positioned in the apical region of the right ventricle. The electrode cable had been passed subcutaneously to the right groin, where it went out through the skin together with the cable of the indifferent electrode placed in the fatty tissue of the abdominal wall (16). I all patients except one (no. 3 series II) a P wave-sensing electrode had been implanted by means of mediastinoscopy (8) with its tip close to the atrial wall and its cable also draw subcutaneously to the right groin, where it passed out through the skin. I the above mentioned patient P waves of an amplitude sufficient to trigger an atrial synchronous pacemaker were obtained with an oesophageal electrode during the study

The studies of series I include recordings of central pressures and Q at rest and during work, with the patients in recumbent position connected to pacemaker p ing a fixed rate of around 70 impulses/min (Siemens-Elema, EM 138). After resting for more than 30 min the patients were connected to an ATP (Siemens-Elema, EM 141) and the same procedure was repeated

The patients in series II were investigated in the same way at rest and during work In thi series however the patients were first connected to th ATP and in the second half of the investigation the FRP was matched to the same impulse frequency as the ATP had given during the determination of Q both at rest and during work.

The choice of work load in both series was governed by the patients performance during one or two work tests performed during the days before catheterization

The same procedure was followed at every catheterization In local anaesthesia double lumen catheter was introduced through cubital vein to the right heart and pulmonary artery and an arterial catheter was introduced percutaneously through the brachial artery and positioned with its tip in the ascending aorta. The reference level for zero pressure was at the mid-thoracic level In no instance was the pacing electrode dislocated by the catheterization procedure. The pulmonary arterial wedge (PCV) pressure

was used as a measure of left atrial pressure In patients in whom no wedge pressure was obtained the diastolic pulmonary artery (PA) pressure was used since this has been found to be almost the same in patients with normal pulmonary vascular resistance (PVR) (12).

Q was determined according to the Fick principle. The methods used in this laboratory for determination of heart volume (HV) blood gases, and for pressure measurement have previously been described (2). The determination of HV was performed with the patients connected to a pacemaker giving impulses at a fixed rate of 70/min. In most cases the examination was made with the patients in the supine position, irrespective of heart and respiratory cycle without angulation of the roentgen tube according to Kjellberg et al. (13) (Table I).

Total haemoglobin (THb) was determined by the alveolar CO method of Sjöstrand (21) modified by Holmgren (11). Normal values per kg b wt. are 10.23 (S.D ±1 18) for men and 8.07 (S D ±0.85) for women.

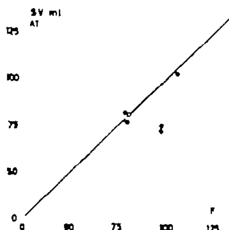


Fig 1 Stroke volume (*SV*) at rest (○) and during exercise (●) and ATP in relation to SV at rest and during exercise at identical loads but with FRP in patients in series I

RESULTS

In series I all patients were studied both at rest and during exercise Patients 2 and 6 who could work without discomfort during FRP got angina pectoris during exercise on the same load during ATP and in no 2 the discomfort was so pronounced that the exercise test was stopped before Q could be determined

In series II patients 2 and 4 were studied only at rest, all the others both at rest and during work No patient felt any discomfort during the investigation.

Series I FRP versus ATP (Table III)

Heart rate (HR)

During ATP the HR at rest averaged 83 beats/min (S D ±11 4) During FRP interference occurred at rest in three patients and the net HR averaged 72 beats/min (S.D ±4.5) During exercise and ATP at a work load averaging 383 kmp/min (S D ±125) which increased the oxygen uptake (V_{O_2}) to an average of 1 034 ml/min (S D ±247.8) the HR increased to an average of 126 beats/min (S D ±11.5) During exercise and FRP interference rhythm occurred in five patients and the net HR was 83 beats/min (S.D ±15 4) V_{O_2} during the two experimental situations was almost identical

Stroke volume (SV)

During ATP the SV at rest averaged 69 ml (S D ±15 1) which is on an average 65% of the normal value predicted from the THb and 54% of the normal value predicted from the roentgenological HV in supine position During exercise there was a significant increase in SV to 81 ml (S.D ±15.5) which is on an average 78% and 66% of the normal value predicted from the THb and HV respectively (Fig. 1)

During FRP the average SV at rest was 72 ml (S D ±10.3) i e not significantly different from the SV obtained at rest during ATP During exercise there was a marked increase in SV to an average of 104 ml (S D ±14.8) which corresponds to *the normal value predicted from the THb (107 ml)* and 82% of the value predicted from the HV The SV during exercise is highly significantly larger than the SV under identical conditions and ATP

Cardiac output (Q̇)

During ATP Q̇ at rest averaged 5 7 l/min (S.D ± 0 9) and increased during exercise to an average of 9 8 l/min (S D ±1 6). During FRP the Q̇ at rest was on an average 5 2 l/min (S D ±0 6) which is almost significantly lower than the Q̇ at rest during ATP During exercise and FRP Q̇ increased to 8.1 l/min (S.D ±0 9) which is significantly less than during exercise and ATP (Fig. 2)

The relationship between Q̇ and V_{O_2} at rest and during exercise is illustrated in Fig 3 During ATP Q̇ is within normal range comparable to that of normal subjects of almost the same age (10) During FRP Q̇ is lower in relation to V_{O_2} than during ATP i.e the same amount of oxygen is transported with a smaller Q̇

Table III *Results in series I (means ±S D)*

	HR (beats/min)	VO_2 (ml STPD/min)	AVD (ml/l)	Q (l/min)	SV (ml)	Pressures (mmHg) Mean PCV or diastol PA	Aortic systol	RV Systolic	End-diastolic	PVR index	SVR index
Rest											
FRP	72 (± 4.5)	258 (± 40.2)	51 (± 7 4)	5.2 (±0.6)	72 (±10.3)	12.5 (± 3.5)	132 (±19.2)	28.8 (± 4 4)	7 4 (±2.1)	2.3 (±0.59)	34.3 (±6.18)
ATP	83 (±11 4)	267 (± 38.7)	48 (± 7.2)	5 7 (±0.9)	69 (±15.1)	9.2 (±4.5)	140 (±20.5)	27 7 (±8.5)	5 6 (±2.4)	2 8 (±1 45)	34.3 (±6.49)
Difference	11	9	3	−0.5	3	3.3	−8	1 1	1.8	−0.5	0 0
	12	12	12	12	11	12	12	10	7	11	12
p	<0 01**	>0.1ns	<0 02*	<0.02*	>0.4ns	<0.01	>0.1ns	<0 6ns	<0 05	>0.3ns	>0 9ns
Work											
FRP	83 (±15 4)	1 042 (±231 1)	127 (±24 1)	8 2 (±0 9)	104 (±14.8)	24.3 (± 6.2)	174 (±17.3)	57 0 (±11.8)	16.3 (±4.3)	2.6 (±1.37)	25 6 (±2.17)
ATP	126 (±11.5)	1 034 (±247.8)	107 (±23 7)	9 8 (±1 6)	81 (±15.5)	23 9 (±10 1)	169 (±26.9)	54 4 (±14 0)	12.7 (±5 3)	2 7 (±1 37)	23 6 (±4 17)
Difference	43	8	20	−1 6	23	0 4	5	2 6	3 6	−0 1	2 0
n	9	9	9	9	10	9	8	9	6	9	9
p	<0 001	>0.7ns	<0 01	<0 01**	<0 001	>0 6ns	>0.3ns	>0.2ns	<0.05	>0 05ns	>0 05ns

ns = non-significant, * $p<0.05$ ** $p<0.01$ *** $p<0.001$

Table IV *Results in series II (means ±S.D)*

	HR (beats/min)	$\dot{V}O_2$ (ml STPD/min)	AVD (ml/l)	Q (l/min)	SV (ml)	Pressures (mmHg) Mean PCV or diastol. PA	Aortic systol.	RV Systolic	End-diastol	PVR index	SVR index
Rest											
FRPm	80 (± 9.0)	263 (± 38 3)	60 (± 9.8)	4.5 (±1.0)	57 (±14 9)	14 1 (±4.8)	136 (±23 7)	30 3 (± 6.5)	8 5 (±3 5)	2 8 (±0.99)	43 1 (±9.6)
ATP	77 (± 7.2)	262 (± 28 6)	51 (± 9 7)	5.3 (±1.3)	69 (±16.2)	12.2 (±4 3)	147 (±26.8)	31 9 (±10 0)	7.6 (±3 5)	2 6 (±1 10)	40.3 (±9 6)
Difference	3	1	9	−0 8	12	1 9	−11	1 6	0 9	0.2	2 8
	12	12	12	12	1	9	11	7	8	8	10
p	>0 05ns	>0.9ns	<0 001	<0 01	<0 01	<0.05	<0 05	>0 4ns	>0.5ns	>0 2ns	<0 05
Work											
FRPm	121 (±17 6)	1 113 (±267.8)	130 (±17 1)	8.5 (±1 6)	72 (±16.9)	28 7 (±5 9)	173 (±18 9)	60 0 (±12 3)	14 7 (±7 0)	3 0 (±1.05)	30.2 (±8 6)
ATP	122 (±19.5)	1 135 (±291 9)	124 (±22 5)	9 2 (±1 8)	77 (±21 8)	28 1 (±7 0)	174 (±28.9)	61 7 (±9 6)	18.– (±6 1)	3 3 (±1 63)	27 6 (±7 4)
Difference	1	−22	6	0 7	5	0 6	1	1 7	3 5	−0 3	2 6
n	11	11	11	11	11	10	9	6	6	9	10
p	>0 3ns	>0 3ns	>0.05ns	<0 05	>0.1ns	>0 7ns	>0 1ns	>0.5ns	>0 1ns	>0 7ns	<0 05

Statistical symbols as in Table III

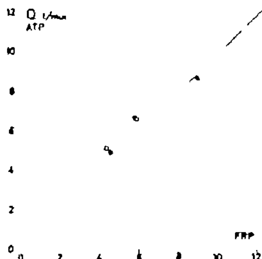


Fig. 2. Cardiac output ($\dot{Q}$) at rest and during exercise and ATP in relation to Q at rest and during exercise at identical loads with FRP in patients in series 1. Symbols as in Fig. 1.

Arteriovenous oxygen difference (AVD)

During ATP at rest the AVD averaged 48 ml/l (S.D. ±7.2) which is higher than in normal young subjects but within the normal range for elderly patients (10). During exercise and ATP AVD increased more than in healthy elderly men. The AVD during FRP at rest averaged *51 ml/l (S.D. ±7.4)*, which is significantly higher than during ATP at rest (p<0.02). During exercise and FRP AVD increased significantly more with Vo_2 than during exercise and ATP.

Filling pressure of the left ventricle (LV)

During ATP at rest the LV filling pressure, as estimated from mean pulmonary wedge (PCW) pressure or diastolic pulmonary arterial (PA) pressure, averaged 9 mmHg (S.D. ±4.5). During exercise it increased markedly to an average of 23.9 mmHg (S.D. ±10.1).

During FRP at rest the filling pressure averaged 1 .5 mmHg (S.D. ±3.5), which is significantly higher than during ATP at rest. In both situations however this pressure is normal. During exercise the wedge pressure *increased to 24.3 mmHg* (S.D. ±6.2) which is not different from the pressure level during ATP and exercise. This increase during exercise observed in both experimental conditions is within the normal range for healthy elderly males reported by Granath et al. (10).

Systemic arterial pressure

The systolic aortic pressure or systolic LV pressure (in patient 12 who had an aortic stenosis) averaged 140 mmHg (S.D. ±20.5) at rest and during ATP. Significant hypertension was not present in any patient. The increase during exercise was normal. During FRP no significant differences occurred compared to ATP. There was no significant difference in aortic mean pressure between FRP and ATP either at rest or during work.

Systemic vascular resistance (SVR)

The SVR index calculated according to the formula

$$\frac{P\,\text{aortic}}{\dot{Q}} \quad \text{BSA} \; \frac{\text{mmHg} \cdot \text{m}^2}{\text{l/min}}$$

(BSA = body surface area) was within normal range both at rest and during work. There was no significant difference in SVR between FRP and ATP.

Filling pressure of the right ventricle (RV)

During ATP at rest the end-diastolic pressure of the RV averaged 5.6 mmHg (S.D. ±2.4) which is within the normal range (10). During exercise it increased to an average of 12.7 mmHg (S.D. ±5.3) which still remains within the normal limits.

During FRP the RV end-diastolic pressure at rest averaged 7.4 mmHg (S.D. ±2.1) which is slightly

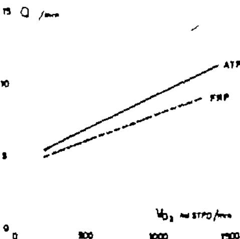


Fig. 3. Cardiac output ($\dot{Q}$) in relation to oxygen uptake (Vo_2) at rest and during exercise in patients in series 1 and ATP (heavy full line) and FRP (heavy broken line). Regression lines for Q on Vo_2 in young healthy men (dotted line) and for elderly men (thin line) with the lower 95% confidence interval (thin broken line).

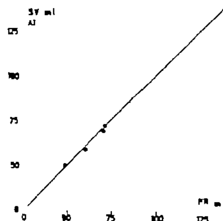


Fig. 4. Stroke volume (*SV*) at rest and during exercise and ATP in relation to SV at rest and during extercise at identical work loads but FRP at the matched frequency (*FRPm*) in patients in series II. Symbols as in Fig. 1

but significantly higher ($p<0.05$) than during ATP. During exercise the end-diastolic pressure rises more and is slightly but significantly higher ($p<0.05$) than during ATP.

Right ventricular and pulmonary arterial pressure

During ATP at rest systolic RV or systolic PA pressure averaged 28 mmHg (S.D. ±8.5) which is within the normal range. During exercise there was a marked increase in the RV systolic pressure (54 mmHg S.D ±14.0), which corresponds to the increase in the left LV filling pressure.

During FRP the systolic RV pressure was not significantly different either at rest or during work.

Pulmonary vasculary resistance (PVR)

The PVR index calculated according to the formula

$$\frac{\bar{P}PA - \bar{P}PCV}{Q} \quad BSA \frac{mmHg \quad m^2}{l/min}$$

was at the upper normal limit both at rest and during work. There was no significant difference in PVR between FRP and ATP.

Series II. ATP versus frequency-matched FRP (FRPm) (Table IV)

Heart rate

During ATP the HR at rest averaged 77 beats/min (S.D ±7.2). During FRPm interference rhythm occurred in three patients and the resulting HR at rest averaged 80 beats/min (S.D. ±9.0). During exercise and ATP at an average work load of 409 kmp/min (S.D. ±157.8) increasing the $\dot{V}O_2$ to an average of 1 135 ml/min (S.D. ±291.9) the HR increased to 1__ beats/min (S.D. ±19.5). During exercise with FRPm interference rhythm occurred in three patients and the resulting HR averaged 121 beats/min (S.D. ±17.6). $\dot{V}O_2$ was almost identical with FRPm and ATP during exercise. With FRPm the $\dot{V}O_2$ averaged 1 113 ml/min (S.D. ±267.8) during exercise.

Stroke volume

During ATP the SV averaged 69 ml (S.D. ±16.2) which is on an average 64% and 54% of the normal value predicted from THb and HV respectively. During exercise there was a significant increase in SV to 77 ml (S.D. ±21.8) which is on an average 71% and 60% of the normal value predicted from THb and HV respectively.

During FRPm the average SV at rest was 57 ml (S.D. ±14.9), which is significantly less than during ATP. During exercise and FRPm SV increased significantly to 72 ml (S.D. ±16.9) which is less but not significantly less than during ATP (Fig. 4).

Cardiac output

During ATP the $\dot{Q}$ at rest averaged 5.3 l/min (S.D. ±1.3) and increased during exercise to an average of 9.2 l/min (S.D. ±1.8). During FRPm $\dot{Q}$ at rest averaged 4.5 l/min (S.D. ±1.0) which is highly significantly lower than at rest and with ATP. During exercise and FRPm $\dot{Q}$ increased to 8.5 l/min (S.D. ±1.6) which is less than with ATP and exer

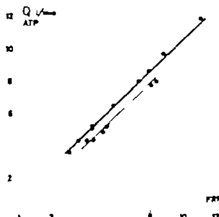


Fig. 5. Cardiac output ($\dot{Q}$) at rest and during exercise and ATP in relation to $\dot{Q}$ at rest and during exercise at identical work loads but FRPm in patients in series II. Heavy line indicates least squares regression for all observations. Other symbols as in Fig. 1

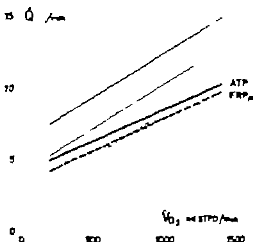


Fig 6. Cardiac output ($\dot{Q}$) in relation to oxygen uptake ($\dot{V}O_2$) at rest and during exercise in patients in series II and ATP (heavy full line) and FRPm (heavy broken line). Other symbols as in Fig. 3.

case on the 5% level (Fig 5). The relationship between $\dot{Q}$ and $\dot{V}O_2$ under these circumstances is illustrated in Fig. 6, from which it is seen that the ATP patients tend to be less hypokinetic

Arteriovenous oxygen difference

The AVD at rest during ATP averaged 51 ml/l (S.D ±9 7), which is higher than in normal young patients but almost within the normal range for ... subjects (10).

...ring exercise and ATP the AVD increased as in patients in series I somewhat more than in ... elderly men (10) and averaged 1 4 ml/l (S D ±22.5). The AVD during FRPm at rest averaged 60 ml/l (S D ±9.8) which is significantly higher than during ATP During exercise and FRPm AVD increased somewhat more but not significantly with $\dot{V}O_2$ than during ATP

Filling pressure of the left ventricle

During ATP at rest the LV filling pressure as estimated from mean PCV pressure or diastolic PA pressure averaged 1... mmHg (S.D ±4.3). During exercise it increased markedly to an average of 28 1 mmHg (S.D ±7.0).

During FRPm at rest the filling pressure averaged 14 1 mmHg (S.D ±4.8), which is almost significantly higher than during ATP at rest. In both situations however the PCV pressure is close to normal. During exercise the wedge pressure increased to 28 7 mmHg (S.D ±5.9) which is not different from the pressure level at ATP This increase during exercise observed in both experimental conditions is only a little higher than in healthy elderly men as reported by Granath et al (10)

Systemic arterial pressure

The systolic aortic pressure averaged 147 mmHg (S D ±26.8) at rest and during ATP The increase during exercise was normal. During FRPm at rest the pressure was somewhat lower than during ATP whereas during work there was no significant pressure difference between the two experimental conditions Significant hypertension was present in two patients (nos. 5 and 10). There was no significant difference in aortic mean pressure between FRPm and ATP either at rest or during work.

Systemic vascular resistance

The average SVR index was high at rest, but decreased to the upper normal level during work. Both at rest and during work the SVR was slightly significantly lower with ATP than with FRPm.

Filling pressure of the right ventricle

During ATP at rest the end-diastolic pressure of the RV averaged 7 6 mmHg (S.D ±3.5) which is within the normal range (10) During exercise it increased to an average of 18.2 mmHg (S.D ±6 1), which is above the normal range

During FRPm the RV end-diastolic pressure averaged 8.5 mmHg (S D ±3.5), which is not significantly higher than during ATP During exercise the end-diastolic pressure rises to the same level as with ATP

Right ventricular and pulmonary arterial pressure

During ATP at rest the systolic RV or systolic PA pressure averaged 32 mmHg (S.D ±10 0) which is only slightly above the normal range of variation. During exercise there was a marked increase in the RV systolic pressure to 62 mmHg (S.D ±9 6), which corresponds to the increase in the LV filling pressure

During FRPm the systolic RV pressure was not significantly different either at rest or during work.

Pulmonary vascular resistance

The average PVR index was at the upper normal range both at rest and during work. There was no significant difference in PVR between FRPm and ATP

DISCUSSION

In the present study atrial triggered pacing has been compared with fixed rate pacing by determination of the central haemodynamics at rest and during work, in patients with AV block selected for treatment with ATP. This implies that all patients had a normal sinus rhythm of the atria. Atrial fibrillation had never been recorded in any patient. Some of the patients had AV block III only during shorter or longer periods. During the study three patients in series I showed interference from conducted sinus rhythm at rest and five during work, when connected to the FRP.

In series II three patients showed interference from conducted sinus rhythm both at rest and during work, when the FRP was connected. This interference between sinus rhythm and pacemaker induced fixed rate certainly influences the results in making the difference in haemodynamic effect of atrial triggered versus fixed rate pacemaking less pronounced.

Another factor influencing the results of the studies is the state of the myocardium. Most patients treated with pacemakers are elderly but for this study rather young patients were selected. The mean age was 56 in series I and 55 in series II. As indicated in Table II many patients had other cardiac lesions with possible myocardial dysfunction and this might also be the case in some of the other patients. This implies that both elastic and contractile properties of the atrial and ventricular myocardium could have been impaired in many of the patients.

This study of the haemodynamic effect of ATP versus FRP is divided into two parts. Series I is a study in which the effect of ATP is compared to the effect of stimulation with FRP at a rate of about 70 impulses/min. Hence the stimulation with ATP implies not only synchronicity between atrial and ventricular contraction, but also an increase in ventricular rate during work. Series II on the other hand is an attempt to study the pure effect of the synchronized atrial contraction on the pump function of the ventricles. The effect of rate in this series is eliminated by adjusting the frequency of the FRP both at rest and during work, to almost the same as that obtained when the ATP was connected. With this planning of the investigation in series II it follows that the study with ATP in every patient had to precede the study with fixed or asynchronous pacemaker. This may introduce a systematic error which, however, is judged to be of minor importance as the patients were allowed to rest for half an hour after the first work test before the next study was performed.

ATP versus FRP at rest

The effect of synchronous versus asynchronous pacing on cardiac output at rest was studied under almost the same conditions in both series.

In series I the average ventricular rate with FRP was 72 and with ATP 83 beats/min, whereas in series II it was somewhat higher with FRPm (80 beats/min) than with ATP (77 beats/min).

On an average the $\dot{Q}$ with ATP is 10% higher in series I and 18% higher in series II as compared to FRP. The significance of the difference is low in series I ($p<0.02$) somewhat higher in series II ($p<0.01$). The difference in increase in $\dot{Q}$ with ATP between the two series could be explained by interindividual variations in a small material. The small differences in HR between FRP and ATP cannot be the explanation, as this should result in a larger difference in series I than in series II. Therefore the increase in $\dot{Q}$ with ATP (10–18%) at rest must be explained by synchronization of atrial systole. This increase in $\dot{Q}$ is in agreement with the results of other authors. Samet et al. (19) demonstrated in six patients that $\dot{Q}$ was about 10% higher during synchronous pacing at rest compared with ventricular pacing, although the interindividual variations were pronounced also in their material.

Wisbeart et al. (23) studied the effect of ventricular and atrial pacing at rest, over a wide range of pacing rates, in 13 patients with stable sinus rhythm after open heart surgery. They did not find any difference in $\dot{Q}$ with atrial pacing at different rates compared to sinus rhythm. With ventricular pacing $\dot{Q}$ was 17% lower than with atrial pacing. As remarked by these authors, the reason for this difference is not only the asynchronicity of atrial contraction with ventricular pacing. With atrial pacing the excitation of the ventricles occurs via normal pathways resulting in a normally coordinated ventricular contraction, while excitation initiated at the implanted ventricular electrode results in an uncoordinated ventricular contraction. In the present study the ventricles were paced with the same ventricular electrode both at ATP and fixed rate pacing thus eliminating the effect of different activation patterns.

The interindividual variations in the effect of ATP on $\dot{Q}$ were large. An example of this is patient

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INITIAL SERUM POTASSIUM LEVEL IN RELATION TO EARLY COMPLICATIONS AND PROGNOSIS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Abstract. During two years 450 patients with AMI have been treated in the CCU at Serafimerlasarettet. Serum potassium level was determined in 444 patients on admission. Hyperkalaemia was a rare finding associated with bad state on admission and a poor prognosis. Hypokalaemia was recorded in 15% of the patients and was associated with previous diuretic treatment, supraventricular bradycardia as well as atrial flutter and fibrillation during the first 24 hours in the CCU Ventricular ectopic beats and ventricular tachycardia were also seen more frequently in hypokalaemic than in other patients.

Cardiac function and arrhythmias in relation to changes in serum potassium concentration have been extensively investigated in experimental and clinical studies Investigations of the incidence of early complications and arrhythmias in relation to initial serum potassium in patients with acute myocardial infarction (AMI) are scarce As treatment with saluretics of patients in age groups with a high incidence of AMI is common, a hypokalaemic tendency in some of these patients may be expected on admission to hospital. An evaluation of early possible and prognostic implications of such findings has been the aim of this investigation

PATIENTS AND METHODS

In 1968 and 1969 450 patients with AMI, 284 men (63%) mean age 63 years, and 166 women (37%), mean age 71 years were treated in the Coronary Care Unit (CCU) at Serafimerlasarettet, Stockholm. The CCU mortality was 10% and the total hospital mortality 21% A detailed description of the CCU as well as the criteria for admission and diagnosis have been presented elsewhere (3) Serum potassium was determined on admission in 444 cases. A conventional technique with autoanalyser was used. The normal values were 3.6–5.1 mEq/l For testing the significance of differences of proportions the χ^2-test was used. almost significant $0.01<p<0.05$ significant $0.001<p<0.01$

Definitions

Left heart failure (LHF). Basal rales a third heart sound or X-ray findings of central vascular enlargement. *Frank pulmonary oedema.* Patients with rales heard all over the chest in association with frothy sputum. *Hypotension* A systolic BP of 90 mmHg or below *Shock.* Hypotension in combination with clinical signs of shock such as cold skin, deterioration of sensorium, oliguria *Supraventricular tachycardia* (SVT). Sinus tachycardia and nodal tachycardia as well as atrial tachycardia, flutter and fibrillation with QRS rate ≥ 100 beats/min. *Supraventricular bradycardia* (SVB). Sinus bradycardia, nodal rhythm or atrial flutter and fibrillation (AF) with QRS rate <50 beats/min *First degree heart block* (A–V I) Sinus rhythm with PQ intervals >0.22 sec. *Ventricular tachycardia* (VT). A rhythm with 3 or more ventricular ectopic beats (VEB) in succession.

RESULTS

The patients were divided into four groups according to their initial serum potassium levels. 64 patients (15%) had <3.6 mEq/l 263 (59%) 3.6–4 3 mEq/l 103 (23%) 4 4–5 1 mEq/l and 14 patients (3%) had a potassium value >5 1 mEq/l Only 4 patients had values <3 0 mEq/l and none >6.0 mEq/l

Hypokalaemia was significantly more common in patients above than below 70 years of age It was also a more common finding in women than in men As could be anticipated, previous medication with diuretics was also significantly more frequent in hypokalaemic (43%) than in normokalaemic patients (27%) Corresponding figures for previous digitalis medication were 35 and 31% respectively

Normokalaemic patients had a total hospital mor

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INCIDENCE AND PRESENTATION OF MYOCARDIAL INFARCTION IN NORTH KARELIA FINLAND

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Abstract A myocardial infarction (MI) register was started on May 1 1972 in the county of North Karelia in Eastern Finland as part of the North Karelia project. Information about all cases with suspected acute MI among the North Karelian population are collected by the register The principles of the register follow the recommendations of the WHO expert working group. Altogether 713 cases were registered between May 1 and Dec. 31 1972. The distribution of them into diagnostic categories was: definite 57% 'possible 30% "no acute MI" 8% and "insufficient information 5%. Patients with no acute MI have been excluded in the results. About 47% of the male patients below 65 years had history of previous MI. Most of the patients in the group had been heavy cigarette smokers, eating mainly butter as the fat in their diet. Overweight was rare among the male patients but not among the females. During the years preceding the attack, most of the patients had visited physician and pathological ECG had been recorded. The average time delay before hospital treatment was internationally relatively short. The 4-week fatality rate among patients below 65 years was 37% for males and 35% for females. These rates were slightly lower than those in the register material in Helsinki. The annual incidence rat per thousand for the age group 30–64 in North Karelia was 13.8 among males and 2.6 among females. The incidence rat increased continuously with age among males, among females it increased markedly only after the age of 60. The risk ratio between North Karelia and Helsinki for the age standardized incidence rates of males in the age group 30–64 was 1.38 and for respective mortality rates 1.21 Within North Karelia the highest incidence rate for males aged 30–64 was recorded in the rural area of Ilomantsi–Tuupovaara in the East.

The high morbidity and mortality rates of coronary heart disease (CHD) in Finland in general and particularly among the middle-aged men living in the county of North Karelia, Eastern Finland, have been known since the 1950s (7). Since then several statistical calculations and the so-called "Seven Countries Study have confirmed these observations (1 5 6 8 9)

A growing concern among the North Karelian population about the high frequency of CHD resulted in 1971 in a petition from the representatives of the population to the national government to take measures to reduce the burden of the disease After the planning stage and with the WHO s assistance a comprehensive preventive programme of cardiovascular disease (CVD) on a community basis was established. The intermediate objectives of this North Karelia project are a) to reduce the general level of the known risk factors of CVD among the population and b) to promote early diagnosis treatment and rehabilitation of patients with CVD The background, aims and methods of the project have been described elsewhere (10).

From the very beginning of the planning of the project it was felt necessary to establish a register to collect uniform information about all possible cases of acute myocardial infarction (MI) and stroke in the county of North Karelia (180 000 inhabitants). These registers started accordingly together with the intervention on May 1 1972. The experience gained by the WHO and some of the collaborating centres (2, 4 14 16) could be used for the methodology of the MI register

The aim of the MI register of the North Karelia project is to serve the preventive programme by collecting information on 1) the occurrence of the disease the natural history of the disease and the features typical of the patients and 2) the function of the health care system at the occurrence of the disease attacks. The information in the register is used both for continuous planning of the primary as well as secondary preventive programmes and for

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Part of these results have been presented in Puska: "North Karelia project—a programme for community control of cardiovascular diseases" (acad. diss.) Kuopio 1974

Table 1 *Division of the material into diagnostic categories*

	Males				Females			
	≤64 y		≥65 y		≤64 y		≥65 y	
	N	%	*N*	%	*N*	%	*N*	%
Definite	208	60.5	93	60.0	33	47.1	73	50.7
Possible	99	28.8	48	31.0	25	35.7	41	28.5
No acute MI	26	7.6	8	5.2	7	10.0	13	9.0
Insufficient data	11	3.2	6	3.9	5	7.1	17	11.8
Total	*344*	*100.0*	*155*	*100.0*	*70*	*100.0*	*144*	*100.0*

assessing the results. The register serves the evaluation of the project by measuring both the incidence of the disease and some of the intermediate objectives of the programme

THE REGISTER

Information about all cases suspected of MI among the North Karelian population is collected by the register The principles of the register follow the recommendations of the WHO expert working group (16). As soon as possible after notification of a case an *initial record form* is completed. At discharge from hospital or if still in hospital, 4 weeks after the onset of symptoms, a *follow-up record form* is completed For fatal cases *death record form* is also completed Half a year as well as one year after the onset of symptoms postal inquiry is made about the patient s condition.

The register centre is situated at the Central Hospital of orth Karelia which treats about 2/3 of all acute MI pa- t in the county A fulltime nurse completes the record of all MI patients treated at the Central Hospital. The maining MI patients are treated at small hospitals mainly attached to local health centres. At each local hospital one or two appointed nurses complete the record forms of all their MI patients. The local hospitals send their record forms immediately to the register centre together with more detailed information on the diagnostic criteria (pain, enzymes and ECG) and the diagnosis established by the treating physician At the register centre the internist of the register (H M.) checks all the record forms and confirms the diagnostic conclusions

The nurse of the register centre keeps in close contact with the local register nurses and checks periodically all death certificates of the county She receives information from the local health insurance office and the hospital dismissal cards of the patients treated for CHD. Health workers of the county are continuously informed about the principles and results of the register through the general information on the project to these workers.

According to the principles of the WHO expert group and the collaborating centres, the cases are divided into the diagnostic categories "definite "possible" "no acute MI" or "insufficient data" The last category consists of some dead patients in whom acute MI as the cause of death can be neither confirmed nor ruled out on the basis of available information The diagnostic conclusions are based on information about pain history enzyme tests, ECG and autopsy (14 16).

MATERIAL

The material consists of the patients registered during the first year of the register i e in whom symptoms started between May 1 and Dec. 31 1972 Of the 713 cases registered during this period 57% fell into the diagnostic category definite 30% into "possible 8% into "no acute MI" and 5% into insufficient data" (Table I) The category no acute MI has always been excluded in the final analysis, the category "insufficient data has been included only when results concerning the incidence mortality and fatality rates are presented. The material includes all cases of acute MI first infarctions and reinfarctions, in the community during the investigation period Twenty-three persons had had an attack twice and two persons three times during the period

For the categories "definite" and possible a pain history was available in 83% an ECG in 74% enzyme test results in 70% and an autopsy diagnosis in 11% of the cases which is 27% of the dead patients. The missing information was usually due to sudden or early death Of this available information the results were considered to be typical of acute MI for pain in 86% for ECG in 38% for enzymes in 76% and for autopsy in 94% of the cases

In about 2/3 of the cases the first source of notification was the arrival in hospital. In the event of such an attack, most patients in North Karelia come directly to the hospital. About 1/3 of the patients were dead before registration. Eighty per cent of the patients came from rural areas. The male patients below 65 years were mainly farmers or lumbermen (39%) industrial workers (25%), or pensioners (21%).

RESULTS

Previous history and risk factors

Among the males 47% of those below 65 years and 50% of those above 65 had had a previous MI most of them only one For the females the attack was

Table II. Fatality rates during the periods from the onset of symptoms to the first medical examination and the 28th day

	Fatality rate (%)			
	Males		Females	
Period	≤64 y	≥65 y	≤64	≥65 y
From the onset to				
The first medical examination	26	32	25	28
The 28th day	37	60	35	49
Total	318	147	63	131

more frequently their first. About 70% of the younger males had suffered from previous angina of effort, 20% from diagnosed hypertension and 7% from diabetes. These data were based on the available information given by the patients and not always confirmed by the records or by a physician.

A positive family history of MI or stroke was more common among younger than older patients. Among the younger male patients it was more common on the father's than on the mother's side (45%/25%). For the female patients the ratio was reverse (31%/43%).

At the time of the attack 65% of the younger male patients were current smokers, a percentage which is clearly higher than that for the general male population of the same age in North Karelia (11), 11% of them had never smoked regularly. The smokers smoked on an average about 20 cigarettes a day. Smoking was much less frequent among the younger female patients (20%). Among the younger male patients 70% had used mainly butter as the fat in their diet. Overweight expressed by a "body mass" index of at least 31 was present in only 8% of the younger male patients compared with 35% of the younger female patients.

About 2/3 of the younger male patients had visited a physician at least six times during the previous three years. Only 6% of them had not visited a physician at all during that period. The blood pressure had been measured during the previous three years in 86% of the male and in 94% of the female patients. Younger females had more often than younger males been told that their BP was elevated (58%/22%). An ECG had been recorded in 83% of all patients. 68% of them had been informed of its abnormality.

Onset of symptoms and delay in medical care

Information about prodromal symptoms 28 days before the onset of the attack was collected by the register. In 68% of the male patients below 65 years the common prodromal symptom was fresh or exacerbation of previous angina of effort and more than half of these patients had felt discomfort in the chest and heaviness in the arm. Among the younger female patients the most common prodromal symptom was discomfort in the chest (85%). In the representative sample of the general North Karelian population at the baseline survey of the project about 2/3 of both males and females aged 45–59 stated that they had angina or discomfort of effort and the same proportion had experienced pain or discomfort in the chest during the previous year (11). About 70% of the attacks started at home. Less attacks (19%) were recorded to have started during the first six hours of day than during the other hours. No major differences were found between the frequency of attacks and the month of onset.

Information about the delay in receiving medical aid was collected in the record form. In these items a lot of information was however missing due to the large number of early deaths (23% of the younger male patients had died before the first medical examination) and in several cases it was difficult to determine the exact time of the onset of symptoms (either because of the gradual start or the nervousness of the family). Thus among the younger male group information was missing about "patient delay" in 53% and about "delay due to conditions" in 38%.

From the information obtained it was calculated that the median delay for the younger male patients in summoning medical aid ("patient delay") was less than two hours. The median delay from summoning medical aid to arrival in the hospital ("delay due to conditions") was for the same group slightly less than one hour. About half of the patients were transported by ambulance and the other half by taxi or private car.

The "patient delay" for younger male patients was less than two hours in 59% of all cases (on whom information was obtained), in 57% of the reinfarctions and in only 48% of the cases with worsening angina as a prodromal symptom. Thus previous infarction did not apparently shorten the delay in summoning medical aid, angina of effort seemed even to lengthen the delay in some cases.

Table III *Annual age and sex specific incidence rates per thousand inhabitants*

	Males		Females	
Age (y.)	*N*	Incidence rate	*N*	Incidence rate
30–34	3	0.9	1	0.3
35–39	5	1 4	1	0.3
40–44	22	5.8	5	1 4
45–49	61	17.8	6	1.6
50–54	59	19 9	11	3..
55–59	72	24.6	16	4 6
60–64	96	33 4	23	6.8
65–69	67	33 7	48	18 4
70–74	48	37.2	43	23.2
75–79	21	32.3	20	18 4
80–	11	27 7	20	27 0
Total	465	14 9	194	5.8

Prognosis

Death before first medical examination was frequent in all groups (Table II) The 4-week fatality rate even if high in the younger male group was hardly of the European average and below the 42% fatality rate in the Helsinki register (12 15).

Nearly half (47%) of all younger male patients had been seen by a physician during the fortnight preceding death Nearly all of them had seen a physician several times during the previous three years. An ECG had been recorded in 91% of the same younger fatal male group 73% of the whole up had been told it was abnormal Previous arction, angina of effort and smoking were more common in the fatal group than in the total material of males below 65 years

A *multiple discriminant analysis was performed* in the group of male patients below 65 years of age with the diagnoses definite or 'possible acute MI to find out whether it might be possible to differentiate the group of survivors from the group of fatal cases on the basis of the existing information on the situation before the attack. Eight variables were accepted in the analysis. When only those cases were included who provided information on all eight variables, the number of cases in the survivor group was reduced from 201 to 184 and in the fatal group from 106 to 66 This analysis showed that the total discriminatory power of the chosen variables was good ($F(8.241)=5.256$). The two variables that predicted the death best were previous ECG (pathological) and previous MI in the history (positive) Working in the same direction, although less markedly were smoking and use of butter Age overweight (body mass index) and previous angina of effort had practically no discriminatory power In this material a history of hypertension was slightly more common in the non-fatal than in the fatal group.

Incidence and mortality

In the calculation of the incidence and mortality rates official population data from Dec 31 1971 were used. These data include corrections on the basis of the national census in 1970. The age- and sex-specific incidence and mortality rates are shown in Tables III and IV The incidence rate among the males increased gradually with age among the females there was a sharp increase only in old age

The incidence and mortality rates have been compared with the results of the MI register in Helsinki (13) Because of differences in age structures of the two populations, age standardization was made using the North Karelian population as standard. The annual incidence rates per thousand for males aged 30–64 were 13 8 for North Karelia and 10.0 for Helsinki, the risk ratio being 1.38 The respective figures for mortality rates were 5 1 and 4 2, the ratio being 1.21

The incidence rates in North Karelia for the different health centre areas were calculated. Although there is considerable random variation due to the rather small absolute numbers, the differences were marked. The urban capital of the county had the annual rate of 12.1 ($N=53$) and the rural area of Ilomantsi–Tuupovaara in the East had the rate of 21 8 ($N=42$) for males aged 30–64 calculated per

Table IV *Annual age and sex specific mortality rates per thousand inhabitants*

	Males		Females	
Age (y.)	*N*	Mortality rate	*N*	Mortality rate
30–34	–	–	–	–
35–39	2	0.6	1	0.3
40–44	8	2.1	1	0.3
45–49	19	5 4		0.5
50–54	23	7.8	5	1.5
55–59	28	9.6	5	1 4
60–64	38	13.2	9	2.7
65–69	39	19.6	20	7 6
70–74	29	22.5	18	9.8
75–79	14	1 6	11	10 1
80–	6	15 1	15	20.3
Total	205	6.6	87	2.6

thousand inhabitants The latter area is the Eastern Finnish area in the "Seven Countries Study" (6).

DISCUSSION

The MI register as part of the comprehensive community preventive programme of CVD serves the programme in several ways as described in the introduction. This article concerns the first year of the MI register of the North Karelia project The emphasis is accordingly on the feasibility coverage comparability and description of the baseline situation.

The MI register in North Karelia covers—at least compared with the registers collaborating in the WHO study—a great geographical area (18 000 km²) with a low population density This caused some worry about the feasibility of the register at the planning stage. The experience is however encouraging. Collection of information does not require very great resources. Integration with the official health services and the use of the information channels of the project are obviously of great help So is the rather low mobility of the population. On the other hand much remains to be done to increase the quality and quantity of the information in many cases, to increase the number of autopsies and to improve the diagnostic remarks in medical records and death certificates. Also it is relatively laborious to build up a comprehensive information system where the information of the MI register is linked with other sources of information on a continuous and personal basis.

The coverage of the register is considered to be good. The well followed recommendation is that all patients suspected of an acute MI are treated in hospital. Cooperation between the register nurse and the responsible nurses at the local hospitals is continuous. A great majority of the cases are registered soon after the attack. In about 15 % of the cases the death certificate is the source of information. Other sources of ensuring the coverage have so far yielded only a few cases.

For the comparability of the diagnostic conclusions inside the county all the registered cases are reviewed and final conclusions are made by the register physician. For the comparability with other centres the principles of the WHO working group are followed as carefully as possible (16). People responsible for the register have been in contact with the WHO and its collaborating centres especially the Finnish ones The register nurse had training at another Finnish centre prior to the start of the registration.

These register results seem to confirm the previous data on the high MI mortality and incidence rates in North Karelia. The age standardized incidence rate among the middle-aged males seems to be nearly 1 4-fold compared with that of Helsinki which had the clearly highest figure in the collaborating centres of the WHO study (16 17) The picture does not change when the comparison is restricted to cases with definite diagnosis of acute MI the definite cases calculated as a percentage of all cases are about the same in the two register materials (65 % vs. 63 %).

The finding is new that the 4-week fatality rate seems to be an exception in the generally unfavourable natural history of the disease in North Karelia This might of course, be due to a registration of more numerous milder cases than e g. in Helsinki on the other hand, because of the distances and the general assumption that the North Karelian men do not easily consult a physician, this might not be true. A lower fatality rate could be explained by the general high physical activity of North Karelian men who mainly have a physically heavy occupation (3).

Preliminary results seem to show that there are considerable differences in incidence rates in North Karelia, the rate being generally higher in the rural areas This would be in agreement with the finding that the general high level of the CHD risk indicators is even higher in rural than in urban North Karelia (11)

The results have several implications in the preventive programme. The high level of the primary risk indicators, frequent heavy smoking and consumption of animal fat, is in agreement with the objectives of the primary preventive programme (10). The findings of the history of morbidity frequent medical contacts and diagnosed coronary symptoms lay emphasis on the intensification of the preventive work by the general practitioners at the local health centres. The high number of reinfarctions lays special emphasis on the secondary preventive programme.

Less benefit in the programme might be expected as to the treatment of the acute phase. About 2/3 of the cases and usually the younger as well as the complicated cases are treated at the Central Hospital with a coronary care unit. Simultaneously the median time delay in transferring the

hospital is remarkably short, especially considering the long distances This, of course does not mean that shortening of the time delay is not necessary and possible in many cases The internationally relatively short average delay seems to be due to the prevailing system in which most emergency patients of this type are transferred directly to the hospital The rather short patient delay might also be due to the high awareness of the problem among the population. On the other hand, due to the high morbidity the various prodromal symptoms of the patients are also very common among the general population of the county according to the surveys made by the project. This reduces the value of the prodromes in the preventive programme

The registration in North Karelia continues As the number of registered cases increases more detailed epidemiological analyses can be made concerning the occurrence of MI in North Karelia. When these data are linked with those from the baseline survey of the project (representative sample of the North Karelia population) conclusions can be drawn about the risk indicators among this population. Simultaneously the register will show during the following years trends in the parameters that are subject to the intervention and thus give feedback to the programme.

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SUBSTRATE INCORPORATION INTO HEPATIC LIPIDS AND PROTEINS IN VITRO IN PATIENTS WITH PRE-β HYPERLIPOPROTEINEMIA

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Abstract Fifty-three patients operated on for uncomplicated gallstone disease have been studied concerning the hepatic synthesis rate in vitro of glycerides and proteins Thirteen of the patients had pre-β hyperlipoproteinemia. Five of them and four normolipoproteinemic patients were fed sucrose-enriched diet for two weeks prior to the operation. In the non-sucrose-fed hyperlipoproteinemic patients the liver concentration of triglycerides (TG) and the incorporation rate of precursors into TG were increased A significant correlation was found between the synthesis rate of TG in liver tissue and the plasma TG concentration in these hyperlipoproteinemic patients. After sucrose feeding of patients with hyperlipoproteinemia the concentration of phosphoglycerides (PG) the incorporation rate of labelled precursors into PG were significantly lower than in normolipoproteinemic patients and in hyperlipoproteinemic patients on an ordinary diet The incorporation rate of leucine into hepatic proteins and the hepatic protein concentration were the same in non-sucrose-fed controls, sucrose-fed and non-sucrose-fed hyperlipoproteinemic patients. The results indicate an increased vulnerability of the hepatic PG and protein metabolism for dietary sucrose in patients with pre-β hyperlipoproteinemia.

The role of the liver in the development of the increased plasma concentration of very low density lipoproteins (VLDL) in patients with endogenous hyperlipoproteinemia has been debated (5 8 10 11 18, 21 22). In a recent study we obtained some evidence for an involvement of the hepatic lipid metabolism in pre-β hyperlipoproteinemic patients (6) Sucrose feeding of these patients was associated with significantly lower capacity for hepatic phosphoglyceride (PG) synthesis However whether this changed hepatic lipid metabolism was an inherent metabolic error in the liver of these patients specifically provoked by the sucrose diet could not be settled.

More recent findings of an altered hepatic lipid and protein metabolism in patients with extrahepatic cholestasis provide further evidence for a contributive role of hepatic metabolism in dyslipoproteinemic conditions (25) In the cholestatic patient with increased concentration of low density lipoproteins (LDL) in serum, the synthesis rate of PG and proteins in liver tissue was increased

The purpose of the present study was to further elucidate the role of the liver in pre-β hyperlipoproteinemia. Hyperlipoproteinemic patients were compared with normolipidemic patients with respect to the capacity in vitro of liver tissue for lipid and protein synthesis Some of these patients were fed sucrose-enriched diet for 14 days before the investigation.

MATERIAL AND METHODS

Clinical material. The study group consisted of 13 patients, 7 men and 6 women aged 55 (±3) years The patients, who all had pre-β hyperlipoproteinemia (types II B and IV) according to Fredrickson and Lees (12) and Beaumont et al. (2), were admitted to the hospital for operation of uncomplicated gallbladder disease and in one patient for atherosclerotic abdominal aortic disease. Clinical data pertinent at the time of admission are given in Table I The plasma triglyceride (TG) and cholesterol concentrations were significantly higher than in randomly selected men, born in 1913 and living in the same city as the study group (4). Five of these patients (nos 9–13) were fed sucrose-enriched diet for 14 days before the operation according to our previous description (6).

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Table I *Clinical data on the study group at the time of admission*

Pat. no	Age (y)	Sex	B wt. (kg) / height (cm) − 100	Plasma TG (mmol/l)	Plasma cholesterol (mg/100 ml)	Lipoprotein type
1	62	♂	1 16	3 4	271	IV
	50	♂	1.38	3.2	319	IV
3	58	♂	1 08	2.7	314	IV
4	72	♂	0 93	2.7	335	IIB
5	61	♀	1 02	2.3	369	IIB
6	50	♂	1 03	3 0	281	IV
7	60	♀	1 13	2.5	340	IIB
8	61	♂	1 19	.5	283	IV
9	24	♀	0 78	.0	215	IV
10	60	♂	1 11	2.6	295	IV
11	51	♀	1.29	.2	211	IV
12	51	♀	1 07	3.8	393	IIB
13	57	♀	1 13	.2	.55	IV
Mean±S E.			1 10±0 04	2.7±0.2	299±15	

As controls served 40 patients 11 males aged 54 (±3) years and 29 females aged 49 (±3) years all admitted to the hospital for an operation of uncomplicated gallbladder disease. Four of the controls (nos 14–17) were fed *sucrose-enriched diet for 14 days before the operation.*

All patients in the study group had normal liver function according to our previous definition (6) None of the control patients had any known disorder apart from the gallbladder disease Patients with known or apparent alcohol problems and women taking oral contraceptive steroids were excluded from the study The plasma TG (1.3±0.1 mmol/l) and cholesterol (217±12 mg/100 ml) concentrations in the control group did not differ from middle-aged men and women from the same city (3) No statistically significant difference was found in relative y weight between the study group (1 10±0.05) and the ols (1 02±0.04)

The plasma lipoprotein pattern was evaluated from repeated plasma lipid analyses lipoprotein electrophoresis on agarose gel (23) and from α-lipoprotein cholesterol determinations (2) as described previously (6).

Experimental procedures All patients fasted from 8 p.m the day before until the operation at approximately 8 a.m. They were premedicated one hour before introduction of general anaesthesia by means of diazepam and atropine Anesthesia was given as hexobarbital (Evipan®) nitrous oxide oxygen and succinyl choline.

The liver biopsy was performed immediately after the abdominal cavity had been opened as described previously (20). The preparation and incubation of liver slices for determination of the incorporation rate of glycerol and fructose into liver lipids and glycogen we e performed according to earlier descriptions (6 20). For determination of the incorporation rate of leucine into proteins liver

Table II *Concentrations of triglycerides phosphoglycerides glycogen and proteins in liver slices from patients in the study and control group*

Pat. no.	TG (μmol g⁻¹)	PG (μmol g⁻¹)	Glycogen (mg g⁻¹)	Proteins (mg g⁻¹)
Pre β-hyperlipoproteinaemia				
1	13.3	24 2	4 4	59 0
2	19 9	21 1	5.6	54.4
3	59.8	19.8	4 4	56.0
4	6	31 7	2 6	79 6
5				62.6
6	12..	21 1	6.1	60.2
7	58.5	21 1	5 7	54.6
8	11.6	26 4	3.8	71 8
Mean±S.E.	25 9±8 7	23.6±1.6	4 7±0.5	6. 2±3 2
Controls				
Mean±S E.	10.1±2.8	23.3±0 9	3.8±0 6	57.8±1 4
n	14	15	8	20
p	<0 05	n.s	n.s.	n.s.

Table III *Concentrations of triglycerides phosphoglycerides and glycogen in liver slices from patients in the study and control groups after sucrose feeding for 14 days*

Pat. no.	TG (μmol g⁻¹)	PG (μmol g⁻¹)	Glycogen (mg g⁻¹)
Pre-β hyperlipoproteinemia			
9	–	21.5	3 4
10	36.3	1.3	11 1
11	34 9	18.0	10 7
12	138.7	19 0	4 0
13	12.1	18.7	6.7
$\bar{X}$±S.D	55.5±28	19 7±0.7	7_±1.6
Controls			
14	3 0	27 7	4 7
15	4_	20.9	5 7
16	38.2	23 9	3 7
17	56.6	16 8	7.5
$\bar{X}$	25.5	22.3	5 4

slices were incubated for 4 hours in complete amino acid mixture (supplied by AB Astra Södertälje) at final concentration four times that of human serum as described in a previous paper (24).

Analytical procedures The lipid phosphorus determinations were performed according to Bartlett (1) as modified by Svennerholm and Vanier (26) Glyceride glycerol was determined as described by Carlson (7). Neutral lipids and phospholipids were separated according to Gloster and Fletcher (13). Glycogen was determined according to van der Vies (27). Protein was assessed according to Lowry et al (15). Determination of the radioactive incorporation into TG PG into the fatty acid (FA) moiety of glycerides and into glycogen was performed as described previously (20). Determination of the radioactive incorporation into protein was performed according to our previous description (24)

The counting of radioactivity was performed in a Packard-Tri-Carb (3320) liquid scintillation spectrometer Correction for quenching was performed by the external standard method.

Statistical methods The Student' *t*-test was applied for the comparison of mean values of different groups Linear regressions were calculated according to the method of least squares and standard procedures were sed to calculate correlation coefficients. *T*-tests were pplied to check the significance of the regression coefficients.

RESULTS

Hepatic content of lipids glycogen and proteins In patients with pre β hyperlipoproteinemia on an ordinary diet prior to operation the liver tissue may contain more TG than that of controls (Table II) The concentrations of PG glycogen, and proteins did not differ from those of the controls.

The five sucrose-fed hyperlipoproteinemic patients (nos. 9–13) showed high TG concentration 55±28 μmol/g, low PG concentration 19 7±0 7 μmol/g, high glycogen concentration, 7.2±1 6 mg/100 mg, and normal protein concentration (Ta-

Table IV *Incorporation rate of glycerol and fructose into triglyceride phosphoglycerides glyceride fatty acids glycogen nd of leucine into proteins in liver slices from patients on an ordinary diet and controls*

	Glycerol (nmol h⁻¹) g⁻¹				Fructose (nmol h⁻¹) g⁻¹				Leucine (nmol h⁻¹) mg prot.
Pat. no.	TG	PG	FA	Glycogen	TG	PG	FA	Glycogen	Protein
Pre-β hyperlipoproteinemia									
1	329.6	105.6	8.6	11 7	108.2	34.2	19 7	7.2	0.26
2	363.5	91.0	2.7	8 6	85 6	30.2	4 0	17 1	0 13
3	351.8	118.6	5.9	17 7	76 2	42.3	12.3	77 1	0.55
4	197.5	110 0	2.6	18.0	59.5	79.3	5.3	22.0	0.20
5		–	–	–					0 40
6	199 4	78.0	5.8	10 0	77 9	30 4	14 4	7.5	0.59
7	237 0	80 1	2.7	17.8	67 1	23.6	6.2	4 4	0.40
8	228.5	83.5	1.2	21 4	15.2	28 6	2.7	22 9	0.35
Mean±S.E.	258.2±23 1	95.3±6.1	4 2±1 0	15.0±1.8	77 1±6.2	31.2±2	9.2±2.4	18.3±3 1	0 37±0.05
Controls									
Mean±S.E.	209±15	82.6±6 4	2.6±0.3	1_8±0.9	89 9±9 0	23.0±3.0	5.3±0.8	19.8±3 1	0 39±0.04
	16	16	16	4	16	16	16	4	20
p	<0.05	<0.10	<0 10		n s.	<0 025	<0 10		n.

Table I *Clinical data on the study group at the time of admission*

Pat. no	Age (y)	Sex	B wt. (kg) / height (cm) − 100	Plasma TG (mmol/l)	Plasma cholesterol (mg/100 ml)	Lipoprotein type
1	6..	♂	1 16	3 4	271	IV
2	50	♂	1.38	3.2	319	IV
3	58	♂	1 08	..7	314	IV
4	72	♂	0 93	2.7	335	IIB
5	61	♀	1 02	2.3	369	IIB
6	50	♂	1 03	3 0	281	IV
7	60	♀	1 13	2.5	340	IIB
8	61	♂	1 19	2 5	283	IV
9	4	♀	0 78	2.0	215	IV
10	60	♂	1 11	2.6	295	IV
11	51	♀	1.29	2..	211	IV
12	51	♀	1 07	3.8	393	IIB
13	57	♀	1 13	2.2	255	IV
Mean±S.E.			1 10±0.04	2 7±0.2	299±15	

As controls served 40 patients 11 males aged 54 (±3) years and ?9 females aged 49 (±3) years all admitted to the hospital for an operation of uncomplicated gallbladder disease Four of the controls (nos 14–17) were fed sucrose-enriched diet for 14 days before the operation.

All patients in the study group had normal liver function according to our previous definition (6). None of the control patients had any known disorder apart f om the gallbladder disease Patients with known or pparent alcohol problems and women taking oral contraceptive steroids were excluded f om the study The plasma TG (1.3±0.1 mmol/l) and cholesterol (217±12 mg/100 ml) concentrations in the control group did not differ from middle-aged men and women from the same city (3) No statistically significant difference was found in relative y weight between the tudy group (1 10±0 05) and the trols (1.02±0 04)

The plasma lipoprotein pattern was evaluated from repeated plasma lipid analyses lipoprotein electrophoresis on agarose gel (23) and f om α-lipoprotein cholesterol determinations (2) as described previously (6).

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The liver biopsy was performed immediately after the abdominal cavity had been opened as described previously (20). The preparation and incubation of liver slices for determination of the incorporation rate of glycerol and fructose into liver lipids and glycogen were performed according to earlier descriptions (6 20) For determination of the incorporation rate of leucine into proteins liver

Table II *Concentrations of triglycerides phosphoglycerides glycogen and proteins in liver slices from patients in the study and control group*

Pat. no	TG (μmol g⁻¹)	PG (μmol g⁻¹)	Glycogen (mg g⁻¹)	Proteins (mg g⁻¹)
Pre β-hyperlipoproteinemia				
1	13.3	24 2	4.4	59 0
2	19 9	21 1	5.6	54 4
3	59.8	19.8	4 4	56.0
4	6..	31 7	..6	79.6
5		–		6.. 6
6	12.2	21 1	6.1	60...
7	58.5	21 1	5 7	54.6
8	11.6	26.4	3.8	71.8
Mean±S.E.	25 9±8 7	23 6±1 6	4 7±0.5	6.. 2±3.2
Controls				
Mean±S E.	10.1±..8	23 3±0 9	3.8±0.6	57.8±1.4
n	14	15	8	20
p	<0.05	n s	n.s.	n.s.

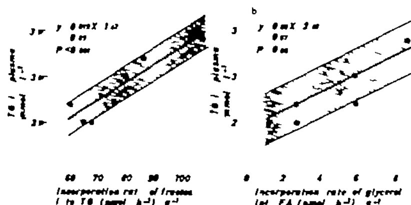


Fig. 1. Correlations between the incorporation rate of fructose into liver triglycerides and plasma triglyceride concentration (*a*) and between the incorporation rate of glycerol into the fatty acid moiety of glycerides (*b*) in liver tissue and the plasma triglyceride concentration in pre-β hyperlipoproteinemic patients on an ordinary diet (*b*). The hatched area indicates ±S.D.

above the upper 95% confidence limit of the non-sucrose-fed controls.

Correlations. In the pre-β hyperlipoproteinemic patients on an ordinary diet bivariate regression analyses showed a correlation between the incorporation rate of fructose into hepatic TG and the plasma TG concentration (Fig. 1*a*). There was also a correlation between the incorporation rate of glycerol into the FA moiety of glycerides and the plasma TG concentration (Fig. 1*b*). The corresponding correlation for the fructose incorporation into the FA moiety of glycerides showed a trend to significance ($r=0.61$, $p<0.10$). No correlation was found between the incorporation rate of precursors into hepatic TG and the TG concentration in the plasma or the relative body weight in the controls.

DISCUSSION

The hepatic production and secretion rates of VLDL in hyperlipoproteinemic patients have been estimated by several methods (5, 8, 10, 11, 14, 17, 18, 21, 22). Some of these studies have indicated that the liver is of major importance for the hypertriglyceridemia in these patients (10, 11, 18, 21), but others have arrived at an opposite conclusion (5, 8, 14, 22). These contradictory results can probably be ascribed to more or less pronounced methodological errors, as recently pointed out by Nikkilä and Kekki (18). The present approach to the problem was an effort to determine directly the hepatic capacity of patients with pre-β hyperlipoproteinemia to synthesize TG, PG, FA and proteins. This in vitro method is for obvious reasons also affected with limitations, particularly concerning the interpretation of the results in a quantitative sense. However, the conditions for the determinations are identical for the study group and the controls, so that qualitative differences should be adequately disclosed.

The present studies showed only slight differences of the hepatic lipid and protein metabolism in patients with pre-β hyperlipoproteinemia as compared with normal controls. More pronounced differences were found when pre-β hyperlipoproteinemic patients on an ordinary diet were compared with those fed a sucrose-enriched diet prior to the liver tissue sampling. In the former the TG concentration in liver tissue was increased. This increase would suggest an enhanced hepatic synthesis of TG. The finding of a higher incorporation rate of glycerol into TG in these patients supports this hypothesis, as does the correlation between incorporation rate into TG and plasma TG concentration. These findings agree with those of Olefsky et al. (21) and Nikkilä and Kekki (19) of a higher TG turnover (measured by endogenous ³H glycerol labelling technique) in patients with endogenous hypertriglyceridemia. Based on their observations Nikkila and Kekki (19) suggested that the primary defect in most cases of type IV endogenous hypertriglyceridemia is an increased inflow transport of plasma TG. However, in our recent study on the effects of a sucrose-rich diet on

the hepatic lipid synthesis and the plasma removal capacity (6) we obtained evidence for both an increased hepatic production of TG and a decreased removal rate from plasma.

The most intriguing finding in our previous study (6) on the effects of sucrose feeding in pre-β hyperlipoprotememia was a lower hepatic PG content and a reduced capacity for hepatic PG synthesis. This finding was confirmed in a small group of patients in this study Moreover the present studies showed that in the hyperlipoprotememic patients on an ordinary diet the capacity for PG synthesis was enhanced. The interpretation of these findings may be that the hepatic PG metabolism in patients with pre-β hyperlipoprotememia (often referred to as carbohydrate-induced hyperlipemia) is specifically vulnerable to sucrose-rich diet To what extent this metabolic error in the liver contributes to the dyslipoprotememia in these patients cannot be decided from the present results

In patients with hyperlipoprotememia, the hepatic protein synthesis as evaluated by the incorporation rate of leucine into soluble liver proteins, did not differ from that of controls. However in the control patients who were fed a sucrose-rich diet prior to the investigation the incorporation rate of leucine was high as compared with non-sucrose-fed controls. However this small case series does not permit any unambiguous conclusion concerning the effect of sucrose feeding on the hepatic synthesis of proteins In the rat it has been shown that the lipoprotein synthesis is enhanced by long-term glucose feeding (9) A stimulation of protein synthesis an increased level of plasma insulin may explain such an effect of carbohydrate diet (16) The lack of response of the hepatic protein synthesis rate to sucrose-rich diet in the patients with hyperlipoprotememia may be another aspect of increased vulnerability of the hepatic metabolism for dietary sucrose in patients with pre-β hyperlipoprotememia.

ACKNOWLEDGEMENTS

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ANTIBODY TITRE CHANGES AND SKIN REACTIVITY IN PATIENTS WITH LIVER CIRRHOSIS UNDERGOING PORTOCAVAL SHUNT OPERATION

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Abstract. Ten patients with liver cirrhosis undergoing portocaval shunt operation have been followed immunologically during their postoperative course regarding antibody titres to various antigens, viral as well as bacterial. The antibody determinations included rubella, vaccinia and cytomegalo viruses, diphtheria toxoid, Candida albicans, streptolysin O, typhoid and paratyphoid O and H and the syphilis reactions: Kahn, Wassermann and Meinicke. Twenty-one blood donors served as controls. Skin test reactions to diphtheria, Candida albicans, streptokinase and tuberculin were performed on the same patients. Eight patients submitted to cholecystectomy served as controls for pre- and postoperative skin tests and antibody titres. The liver cirrhosis group before operation had a significantly higher number of elevated antibody titres concomitant with a significantly reduced skin test reactivity to diphtheria toxoid and streptokinase. An increase in the number of elevated antibody titres was seen after portocaval shunt operation. In no case was a higher antibody titre associated with an increase in skin reactivity to the corresponding antigen. A number of significant titre changes to viral antigens were seen in the postoperative course without clinical evidence of the corresponding viral disease. These findings indicate that under certain circumstances antibody titre changes should be interpreted with caution.

The influence of the liver on antibody titres and γ-globulin levels has recently been discussed by several authors. The following causes of hypergammaglobulinemia and high antibody titres have been postulated: 1) Non-specific altered globulin production in the diseased liver may give rise to non-specific elevated antibody titres (3, 4, 21). 2) Decreased activity of Kupffer cells due to damage of the liver and/or lowered blood flow through the liver could explain the rise of antibody titres because of larger amounts of circulating antigens not phagocytized by the liver (6, 30). 3) Lowered immunosurveillance, resulting in increased growth and reduced clearance of microorganisms, may increase the antigenic load presented to the antibody forming system (6, 19).

The present investigation has two purposes: 1) to follow antibody titres to common antigens in patients with liver cirrhosis undergoing portocaval shunt operations, 2) to estimate cell-mediated immunity (CMI) in these patients on the basis of skin tests.

PATIENTS

Sera were collected from patients with alcoholic and postnecrotic liver cirrhosis within an age range 22–67 years (9 males and 1 female). Diagnosis was confirmed by liver biopsy. All patients had suffered bleeding from oesophageal varices and were scheduled for surgery at the time the first sera were obtained. Twenty-one blood donors, aged 23–62 years (18 males and 3 females), served as controls.

Eight patients, aged 19–65 years (3 males and 5 females) undergoing cholecystectomy, served as controls for skin test reactions as well as for antibody titres to diphtheria toxoid and Candida albicans precipitins. All controls had normal γ-globulin levels.

METHODS

The sera were examined for haemagglutination-inhibiting (HI) antibodies to rubella (12) and vaccinia viruses (16), and complement-fixing (CF) antibody to cytomegalo virus (CMV) (1).

Antistreptolysin O (AST) was determined according to Kalbak (15), antistreptococcal hyaluronidase (ASH) according to Faber (9), diphtheria toxoid (HI titre) according to Scheibel (23) and Candida precipitin score according to Axelsen et al. (2). Syphilis reactions included Wassermann (WR), Meinicke (MR) and Kahn tests (25). The

Table I. *Comparison of the incidence of high antibody titres in cirrhotics before operation and in controls*

	AST ≥160	ASH ≥7	Candida ≥3	Diphtheria ≥0.3	Typhoid-paratyphoid PO ≥50	TO +	PH +	TH +	Syphilis Kahn +	MR +	WR +	CMV ≥32	Rubella ≥40	Vaccinia ≥32	Total no. of elevated titres
Cirrhotics	3/10	2/10	4/10	6/10	2/10	0/10	0/10	0/10	0/10	2/10	0/10	2/10	7/10	8/10	36/140
Controls	0/21	0/21	1/8	0/8	0/13	0/13	0/13	0/13	0/13	0/13	0/10	1/14	5/21	2/13	9/194
P* (%)	2.7			1.1									1.9	0.3	

Fisher's exact test.

was performed for Salmonella typhi O and H (TO, TH) and Salmonella paratyphi O and H (PO, PH) (8).

Skin tests were performed with 0.1 ml Candida albicans extract (1:1000) (Allergologisk Laboratorium, Copenhagen), 0.1 ml diphtheria toxoid B antigen (Statens Seruminstitut 0.5 Lf/ml), 0.1 ml streptokinase equal to 10 IU (Behringwerke) and 0.1 ml purified tuberculin RT23 equal to 1 IU (Statens Seruminstitut, Copenhagen). The diameter of the induration following antigen injection was measured 48 hours after inoculation.

The left thigh was used for initial skin tests before operation, while the right was used for retesting after operation.

The antibody titrations, other than rubella and vaccinia, were performed by Dr H. K. Andersen, Institute of Medical Microbiology, Århus University, Dr N. H. Axelsen, Protein Laboratory, University of Copenhagen and the Diagnostic Bacteriology, the Serum & Vaccine, the Streptococcal and the Treponematosis Departments of the Statens Seruminstitut. The statistical calculations were performed by the Biostatistical Department of the Statens Seruminstitut.

RESULTS

e I shows that rubella HI, vaccinia-HI, phtheria antitoxin and AST titres are significantly higher in cirrhotics than in controls. In addition an increased number of elevated titres against ASH, Candida, PO, MR and CMV are seen in these patients, the total number of elevated antibody titres being 36 of 140 as compared to 9 of 194 in the controls.

Table II shows the results of an antibody titre index calculation for the 10 cirrhotics approximately 1 month after portocaval shunt operation. The calculation is based upon determination of antibody titres to 9 different antigens. It will be seen that 8 of 10 patients had a positive titre index and none were negative. The result is significant ($p<0.004$, Wilcoxon's sign test).

Table III shows an example of titre changes in a single patient after portocaval shunt operation. The patient showed a significant increase in rubella titre from 20 to 160, and an elevation of vaccinia titre from 32 to 256.

Fig. 1 shows the results of skin tests performed preoperatively with Candida albicans, diphtheria toxoid, streptokinase and tuberculin in the group of patients with liver cirrhosis and in the group with gallstones. Skin test reactions to the four antigens used were reduced in the liver cir

Table II. *Titre index in liver cirrhosis one month after portocaval shunt operation*
+1 = increase, 0 = unchanged, −1 = decrease in titre

Antibody	Pat. no. 1	2	3	4	5	6	7	8	9	10	Antibody titre changes: Increases (n)	Decreases (n)
Rubella	−1	+1	−1	+1	0	0	0	0	+1	0	3	2
Vaccinia	−1	0	+1	+1	+1	0	0	−1	0	+1	4	2
CMV	0	+1	+1	+1	0	+1	+1	+1	+1	0	7	0
AST	+1	0	+1	−1	+1	+1	0	+1	+1	0	6	1
ASH	0	0	+1	−1	0	0	0	−1	0	0	1	2
MR	+1	0	0	+1	0	+1	+1	+1	0	−1	5	1
WR	0	0	0	+1	+1	0	0	+1	0	0	3	0
TH	0	0	0	0	0	0	+1	0	0	0	1	0
PO	0	0	0	−1	0	0	+1	0	0	0	1	1
Total index score	0	2	3	2	3	3	4	2	3	0		

Table III A tibody titre changes in patient 4 after portocaval shunt operation

No. of days before or after operation	Ru-bella	Vac cinia	CMV
−8	20	32	4
+10	40	256	8
+17	160	64	16
+60	40	32	8

rhosis group compared to the gallstone group, though significantly only to diphtheria toxoid and streptokinase

In Fig. mean titres to diphtheria toxoid and Candida albicans and results of skin tests to these antigens pre- and postoperatively for 3 cirrhosis patients undergoing portocaval shunt operations are compared with the results obtained from 5 patients undergoing cholecystectomy After portocaval shunt the mean antibody titres increased concomitantly with a decrease in the skin test reactions while no titre changes and a normal increase in induration were observed after cholecystectomy

DISCUSSION

Our results indicate that elevated antibody titres to a number of common bacterial fungal and viral antigens are found in patients with liver cirrhosis

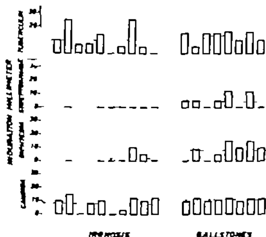


Fig 1 Skin test reactivity to Candida albicans, diphtheria toxoid streptokinase and tuberculin in patients with cirrhosis and patients with gallstones. —mean induration, $*=p<5\%$ $b=p<1\%$ (Wilcoxon s two sample test).



Fig 2 Changes in antibody titre and skin reactivity to Candida albicans and diphtheria toxoid in patients undergoing portocaval shunt operation (mean of 3 patients) or cholecystectomy (mean of 5 patients). Antibody titre Δ pre- and ▲ postoperatively Induration ○ pre and ● post operatively

After portocaval shunt these titres increased and in the postoperative course there were a number of titre changes to the antibodies in question. Depressed CMI is well recognized in many forms of liver diseases (10, 11 17 20). This study shows that skin reactivity to common antigens such as streptokinase and diphtheria toxoid may also be impaired. In organ transplanted patients repeated skin tests to tetanus toxoid failed to produce increased responses contrary to the findings in normal individuals (13) In the three cirrhotics skin tested twice there was a lack of booster effect, whereas none of the five cholecystectomized patients showed a reduced skin reaction to the postoperative skin test.

Circulating autoantibodies (7 14 32) and antibodies to bacterial (3 4 21 30), viral (6) and certain dietary proteins (30) are reported to be increased in liver diseases However high levels of autoantibodies and antibodies to viruses and bacteria are also found under various conditions associated with impaired CMI (1 26)

Recent publications (29 31) have discussed the pronounced hyperglobulinaemia in liver diseases, and an increased amount of circulating antigens as well as an impaired T cell function are suggested as the causative mechanism. We would like to add the suggestion that raised plasma cortisol level due to protein deficient diet (24 27) altered hormone turnover and cortisone treatment (5 4) could lower an already impaired T cell suppressor function and further enhance antibody production (28) Our find

ings of significant titre changes to viral antigens in the postoperative course may be explained as non-specific anamnestic responses or as a response to reactivated infections Since diphtheria antitoxin pertains to an antigen other than a living microorganism a non-specific anamnestic response is likely at least in this case The Kahn, TO and PH tests which are rather specific for the respective infections were negative in all portocaval patients as well as in controls *The raised antisalmonella agglutinins* in 11 of 40 patients and in 9 of 20 patients with liver diseases reported from the USA (71) and Italy (3) respectively are not in accordance with our findings The lower incidence of typhoid and paratyphoid fever in Denmark compared to these countries could however explain the discrepancy

The increase in antibody titres after portocaval shunt is in accordance with the reported increase in serum globulins and antibody titres to Escherichia coli following portocaval shunt operation in both humans and rats (18 22) Whether this is caused primarily by diminished blood flow through the liver or by the strain of major surgery remains to be determined However we would like to suggest that the humoral responses to the operation are to some extent caused by suppression of the CMI leading to reactivation of intercurrent and latent infections

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LIVER IMPAIRMENT DURING CHRONIC HEMODIALYSIS AND AFTER RENAL TRANSPLANTATION

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Abstract Liver impairment has been evaluated in consecutive series of 79 patients with chronic renal failure of whom 23 were treated with hemodialysis alone and 37 with hemodialysis and renal transplantation alone. In half of the chronic hemodialysis patients and half of the patients receiving renal allograft elevation of serum alanine aminotransferases was observed for shorter or longer period during the study In half of these cases from both groups the clinical course, laboratory data and liver histology were consistent with virus hepatitis and four patients died from fulminant hepatic failure. In the other half of the patients with elevated transaminases, this was either asymptomatic and unexplained or due to other causes such as septicaemia or urinary leakage. Liver biopsy showed unspecific changes. Renal transplantation was not performed in patients suffering from virus hepatitis but 12 of the 37 patients who received renal allograft had elevated aminotransferases at the time of transplantation. In seven of them a marked increase in aminotransferase was observed postoperatively but none developed clinical sign of liver disease. It is concluded that elevated aminotransferase activity per se is no contraindication to surgical procedures, including renal transplantation, in these patients. However liver biopsy should be performed to detect possible liver disease.

Liver impairment is often seen in patients treated with chronic hemodialysis and in patients who have received a renal allograft (4 8 9 10 11 13 14 20 23 24). Virus hepatitis has most often been incriminated. Of the 79 patients reported here half of those treated with chronic hemodialysis and half of those treated with a renal allograft showed elevation of alanine aminotransferase activity in serum As surgical procedures may be fatal in patients with virus hepatitis it is important to know whether elevation of aminotransferases in a patient who is going to receive a renal transplant is due to hepatitis. Likewise elevation of aminotransferases in a patient receiving immunosuppressive treatment after transplantation represents a problem as both toxic liver damage and virus hepatitis may be the cause The purpose of the present work is to evaluate the possible causes of elevation of aminotransferases in patients treated with chronic hemodialysis and renal transplantation.

MATERIAL AND METHODS

The material consists of consecutive series of 79 patients, 23 of whom were treated with hemodialysis alone (May 1963 – May 1970), 37 with hemodialysis and renal transplantation, and 19 with allotransplantation alone (Jan. 1968 – May 1970). Among the latter L patients had previously been treated with hemodialysis in other centres

The material is divided into hemodialysis patients ($n=60$) and patients followed after renal transplantation ($n=56$) 37 patients were studied both during hemodialysis and after transplantation, and are thus included in both groups.

The following liver tests were performed at least four times per month, serum alanine aminotransferase, prothrombin-proconvertin and electrophoresis of serum proteins. From Oct. 1968 cytomegalic virus antibody titre in serum and from Nov 1969 Hb-antigen (27) were determined in all patients. Liver biopsies were performed with the Menghini needle. Liver histology either from biopsy or autopsy was assessed in 30 patients

Anicteric hepatitis was suspected in patients with sudden rise in alanine aminotransferases concomitantly with clinical symptoms consistent with virus hepatitis. The diagnosis was verified by histology in half of the cases. Clinically biochemically and histologically the chronic hemodialysis patients and the patients who had received renal allograft could be divided into the following five groups: I) no clinical or biochemical evidence of liver impairment, II) elevated alanine aminotransferases but no clinical evidence of liver disease, III) anicteric hepatitis IV) icteric hepatitis, V) elevated alanine aminotransferases possibly due to other causes than virus hepatitis.

Hemodialysis technique

Three types of dialysers have been used Skeggs-Leonards (25) until April 1964 Kiil (17) until March 1969 and thereafter Gambro disposable dialysers (1).

Table I *Hemodialysis material*

No. of pats.		Observation period (d.)	Hemodialysis* (n)	Blood transfusions* (n)	Liver biopsies or autopsies	Hepatic precoma or coma	Deaths from liver failure	Renal allotransplants
29	Without liver impairment	165 (7 540)	32 (2 242)	41 (4–107)	6			23
9	Asymptomatic alanine aminotransferase elevation	669 (90–1 980)	146 (7–520)	112 (7–307)	2			7
5	Anicteric hepatitis	624 (60–1 260)	162 (17 342)	96 (17 242)	3			3
10	Icteric hepatitis	255 (30–450)	45 (5–97)	40 (12–80)	7	5	3	1
7	Other causes	514 (30–1 890)	148 (15–513)	190 (48–491)	4			3
60	Total				22	5	3	37

Average, range within parentheses.

The first two types of dialysers were used in combination with pumps constructed by Ole Dich Denmark, or a Travenol pump on the blood side The Gambro dialysers have been used in connection with DDS consoles (6, 7). I this system contamination of the equipment can be avoided (7). Cuprophane® pT 150 membranes have been used in the dialysers. Membranes used with the Kil and the _eonards dialysers were boiled for 30 min and –↑ in Rodalon® 1%, phenoxyl 1% or chloramine 5% solution before se. Prior to use of the DDS consoles the machinery was perfused with the same solutions and once week with a 3% acetic acid solution The tubes were gammasterilized PVC for medical use

The patients were dialysed either through an internal Cimino-Brescia fistula or through an external shunt a.m. Sribner-Quinton All patients were dialysed in the hospital for 10–16 hours twice a week. On an average the serum creatinine concentration fell from 12.4 to 4 4 mg/100 ml, and BUN from 127 to 19 mg/100 ml The patients were hospitalized only in connection with shunt problems or intercurrent diseases. Most of the patients were gainfully employed during the treatment.

Renal transplantation

Patients receiving a renal allotransplant were anaesthetized with babiturate-suxamethoniumchloride N_2O-O_2-cyclopropane. Thirty patients were treated with extracorporeal irridation of the blood (28) before transplantation. After transplantation the immunosuppressive treatment consisted of prednisone 40–60 mg daily and azathioprine (Imuran®), 5 mg/kg b wt. on the first day and thereafter approximately 2 mg/kg b wt. daily

RESULTS

Hemodialysis material

The average observation period number of hemodialyses and blood transfusions are given in Table I The fewest number of hemodialyses and blood transfusions are seen among patients without liver impairment and among patients with icteric hepatitis The differences however are not statistically significant.

In 29 patients (group I) no liver impairment was observed during hemodialysis

In nine patients (group II) elevated alanine aminotransferases were observed but no symptom or clinical sign of liver disease was present. Characteristically the enzyme elevation was small (2–4 μmol/h/ml) and lasted for 2–4 months except for one case in whom it persisted for one year Hb-antigen test was negative in eight patients and in one patient is was not performed Liver biopsy was performed in two patients and the liver histology was normal in both.

Five patients (group III) had anicteric hepatitis. The diagnosis was based on a typical clinical course and typical course of aminotransferase elevation Three of the diagnoses were confirmed by liver biopsy Hb-antigen was investigated during the acute phase of the disease in one patient and in four

Table II *Results in hemodialysis patients undergoing renal transplantation (RAT)*

No. of pats.		During hemodialysis		After RAT				
		Elevated alanine aminotransferase at the time of RAT	Peak enzyme elevation 1 mo. after RAT	No liver impairment	Asymptomatic alanine aminotransferase elevation	Anicteric hepatitis	Icteric hepatitis	Other causes
23	Without liver impairment			12	4	4		1
7	Asymptomatic alanine aminotransferase elevation	7	3	2	4	1		
3	Anicteric hepatitis	3	3			3		
1	Icteric hepatitis	1	0		1			
3	Other causes	1	1	1				2
37	Total	12	7	15	9	8	2	3

patients after the acute disease. The antigen was present in three patients. In one of them two liver biopsies performed with an interval of six months showed chronic aggressive hepatitis.

Ten patients (group IV) had icteric hepatitis, six of whom had typical virus hepatitis and four cholestatic hepatitis with high alkaline phosphatases and high serum bilirubin values. Evidence of drug-induced hepatitis was not found, and none of them received azathioprine or corticosteroids.

The liver biopsies were almost identical showing marked centrilobular cholestasis, few liver cell necroses and practically no cellular infiltration. In the other patients liver histology was typical of virus hepatitis, ranging from spotty to total liver cell necroses.

Five patients lapsed into hepatic precoma or coma, and three of them died from hepatic failure. Hb-antigen was investigated during the acute phase of the disease in five of the patients and at various times after the disease in two; all were negative.

Group V comprises seven patients, all without jaundice. Elevated alanine aminotransferases were assumed to be due to bacterial sepsis in two patients, crush syndrome with anuria in two, iron contamination of the dialysis water in one, cardiac arrest in one, and severe hemosiderosis of the liver with focal necroses of the liver cells in one patient.

Cytomegalic virus titres showed no significant rise during the investigation period in any of the patients examined.

Renal allotransplantation material

Thirty-seven of the hemodialysis patients received a renal allograft (Table II). Eleven of 23 patients in group I developed liver impairment, in six due to virus hepatitis. One patient with hepatitis died three months after the renal transplantation in fulminant hepatic failure.

All the patients in groups II, III and IV had elevated alanine aminotransferases at the time of transplantation and in six a marked increase was observed during the first month after transplantation. None of them had jaundice or other clinical signs of liver disease. The patients with virus hepatitis during hemodialysis (3 in group III and 1 in group IV (Table I)) underwent renal transplantation at a time when clinical signs of hepatitis were absent. The shortest period between hepatitis and operation was one month.

In Table III patients treated with renal allografts are divided into the same five groups as the hemodialysis patients and the corresponding figures are given. In conformity with the hemodialysis material, the fewest number of hemodialyses and blood transfusions were seen among patients without liver impairment and among patients with icteric hepatitis.

In 28 patients (group I) no liver impairment was observed. Asymptomatic elevation of alanine aminotransferases was seen in nine patients (group II). Liver biopsy was performed in two patients. One biopsy showed moderate to marked fatty in-

Table III. *Renal allotransplantation (RAT) material*

No. of pats.		Observation period after RAT* (d)	Hemodialyses* (n)	Blood transfusions* (n)	Liver biopsies or autopsies	Hepatic precoma or coma	Deaths from liver failure	Treated with hemodialysis
28	Without liver impairment	175 (30–720)	54 (0–533)	38 (1–277)				23
9	Asymptomatic alanine aminotransferase elevation	340 (30–854)	66 (5–185)	76 (7–163)	2			7
10	Anicteric hepatitis	389 (215–610)	103 (0–342)	94 (12–242)	2			3
4	Icteric hepatitis	189 (120–335)	63 (0–200)	26 (16–38)	3	2	1	1
5	Other causes	231 (90–396)	133 (9–513)	116 (8–496)	1			3
56	Total				8	2	1	37

Average, range within parentheses

filtration and the other a few liver cells with cloudy swelling. In this patient Hb-antigen test was positive. Three other patients in this group were Hb-antigen negative.

Anicteric hepatitis was suspected in ten patients (*group III*) and the diagnosis was verified in two by biopsy. Hb-antigen test was positive in eight and negative in two patients.

Icteric hepatitis was seen in four patients (group V), two of whom lapsed into hepatic precoma or a. As previously mentioned one of them died hepatic failure. Hb-antigen test was positive in e and negative in two patients. The antigen was not determined at the time when the patient with fulminant hepatitis died.

Five patients (*group V*) were assumed to have elevated alanine aminotransferases due to the following causes: one had polycystic kidneys and cysts in the liver (verified at biopsy), three developed alanine aminotransferase elevation in connection with urinary leakage and urinary tract infection, and one had marked hemosiderosis in the liver (the same patient as in the hemodialysis material). Hb-antigen test was negative in two of these patients. Cytomegalic virus titres did not rise significantly in any of the patients treated with renal allotransplantation.

Staff

The staff was checked twice monthly for elevation of alanine aminotransferases. Four members developed hepatitis during the investigation period. All were jaundiced and the clinical course was mild. All these cases appeared before Hb-antigen test could be carried out, but later investigations have revealed no Hb-antigen positive cases among the staff (27).

DISCUSSION

In the present material 50% of the chronic hemodialysis patients and 50% of those treated with renal transplantation had elevated alanine aminotransferases for shorter or longer periods. Outbreak of hepatitis in dialysis centres has been a serious complication in the treatment of chronic renal failure (4, 8, 9, 10, 11, 13, 14, 20, 23, 24), and some centres have been forced to interrupt hemodialysis treatment and renal transplantation for this reason (18). In this centre, hepatitis has been observed throughout the 7-year study period, but no regular epidemic outbreaks have occurred. The increase in the number of patients with hepatitis parallels the number of patients treated with chronic hemodialysis. The mortality from hepatitis was 13%. No case of postnecrotic cirrhosis was seen, but one patient developed chronic aggressive hepatitis. None of the patients received a renal graft during the acute phase of hepatitis, but 12 had elevated aminotransferases at the time of transplantation. In seven of them a short-lasting marked increase in serum enzymes occurred after the transplantation, but none became jaundiced or died. This indicates

that the elevation of alanine aminotransferases is not a contraindication for performing renal transplantation. Death from hepatic failure after renal transplantation has been reported (5 13) and one of our patients died from hepatitis three months after transplantation. This patient had normal alanine aminotransferases and no evidence of hepatic dysfunction at the time of transplantation.

As pointed out by London et al (19) serum alanine aminotransferase is probably the most valuable test for liver disease in patients treated with chronic hemodialysis Many patients receive anticoagulants and the prothrombin concentration is therefore diagnostically useless. Alkaline phosphatases may be elevated due to osteomalacia and/or secondary hyperparathyroidism and albumin may be low due to restricted protein intake Bromsulphalein retention may be of greater value but was not performed in all our patients Many authors have used elevated alanine aminotransferases as a conclusive criterion of virus hepatitis in patients treated with chronic hemodialysis and renal transplantation. We interpret our data so that only half of our patients with elevated alanine aminotransferases suffered from virus hepatitis.

As mentioned by Mattenheimer et al (21) and Bergman et al. (3), however several causes of the hepatic involvement are possible in these patients. Cytomegalic virus infection has been reported during chronic hemodialysis and after renal transplantation (2 9), but cytomegalic virus titres did not rise significantly in any of our patients. Different drugs such as tranquilizers analgetics antibiotics, anticoagulants and immunosuppressive drugs especially azathioprine and corticosteroids, have been given to patients in the present study Liver impairment due to most of these drugs is well known but no evidence of drug-induced liver damage was found in the present series and especially no case of azathioprine cholestasis was seen (26) Liver damage in connection with extracorporeal irradiation of the blood has not been described but has been reported due to split products (esters of ftalyl acid) from the gammasterilized PVC tubes (15 22) and we cannot exclude that this was the cause in some of our patients Enzyme elevations and abdominal discomfort, however were not seen in immediate connection with the hemodialyses Our patients have received a diet containing at least 40–50 g protein per day which should exclude malnutrition as a cause of liver impairment (29). The cause of asymptomatic enzyme elevations in our material remains unknown but some of these patients might have had an asymptomatic and slight hepatitis which however appears to be of no clinical significance. Liver biopsy is valuable in assessing liver disease in these patients and the risk of bleeding following liver biopsy is minimal if the biopsy is taken about 18 hours after stopping hemodialysis, and if protamine sulphate is given at the end of the hemodialysis No complication after liver biopsy performed under these conditions was seen

Hb-antigen determinations were performed only during the last 6 months of this investigation. It is striking however that none of the chronic hemodialysis patients were Hb-antigen positive. Among patients who had received a renal allograft, 11 were Hb-antigen positive but it is not known whether they were positive or negative before the transplantation. In this centre fortunately no serious cases of hepatitis occurred among the staff

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CLINICAL LISTERIOSIS IN RENAL ALLOTRANSPLANTATION

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Abstract Two cases of *Listeria monocytogenes meningitis* among 212 kidney transplanted patients (total of 339 patient years of observation) under immunosuppression with azathioprine and prednisone are presented Both cases developed shortly after an increase of the immunosuppression. The first case appeared in a 27 year-old man 5 days after a minor increase of the azathioprine dose from 75 to 100 mg/day The course was relatively mild and the patient was cured by tetracycline. The second case appeared in a *52-year-old woman 5 days after* a massive increment of the steroid dose and administration of a moderate azathioprine dose carried out to revert a rejection of the graft. This case had a fulminant course and was complicated by Listeria sepsis with hemolysis, pronounced oliguria and *thrombocytopenia* leading to fatal internal bleedings, primarily in the brain. Considering the poor prognosis of this complication it is suggested that cytotoxic drugs are temporarily discontinued and the steroid dose reduced at the height of the infection.

The rather common infection with Listeria monocytogenes is generally asymptomatic in healthy untreated individuals (1).

Clinical listeriosis is usually found in individuals with lowered resistance relating to malignancy (leucaemia, lymphoma, Hodgkin's disease) diabetes mellitus cirrhosis of the liver and/or medication with cytotoxic drugs or steroid hormones (1 2 4 5 11 12, 13) A few cases have, moreover been reported in immunosuppressed patients after renal allotransplantation (6, 7 11).

This paper describes cases of Listeria meningitis among 21 immunosuppressed kidney transplanted persons, who have lived on an average 1 6 years after grafting.

CASE REPORTS

Case 1

A 27-year-old man with chronic glomerulonephritis received kidney transplant from his father (HL-A mismatch in one locus). *Immunosuppression with prednisone and azathioprine was started and after 9 months the patient* was discharged with 4 hour creatinine clearance of 60–70 ml/min, no proteinuria and normal BP At this time he received prednisone, 30 mg/day and azathioprine, 75 mg/day

Five months after the transplantation the patient was readmitted on Jan. 13 1969 after days of intense, diffuse headache pains in the back and the neck, slight dysuria, but no diarrhea. On Jan. 9 the azathioprine dose had been *increased from 75 to 100 mg/day* On admission the patient was relatively unaffected Physical examination revealed only temperature of 38.7°C and slight tenderness, but no stiffness of the neck Hb 15 1 g/100 ml WBC 15 000/mm³ with 89% polymorphonuclear leucocytes, 7% lymphocytes, % monocytes and 2% neutrophil myelocytes, thrombocytes 97 000/mm³ Blood cultures were negative Urinalyses revealed 5–8 leucocytes 5–7 erythrocytes and bacteria. As urine cultures, performed on Jan 9 in the Outpatient Service had grown more than 10^5 Klebsiella pneumonia per ml. treatment with tetracycline was started However during the day the temperature rose to 40 1°C

On Jan. 14 stiffness of the neck and nystagmus to the left was noted Lumbar puncture was unsuccessful due to artifical bleeding. On Jan 15 a new lumbar puncture revealed clear cerebrospinal fluid under normal pressure Analyses gave WBC of 118/mm³ with 70% mononuclear and 30% polynuclear cells Protein was 90 mg/100 ml and glucose 54 mg/100 ml (blood glucose 137 mg/100 ml). Microscopy and culture of the spinal fluid revealed no microorganisms EEG was normal Urine culture from the day of admission had now revealed significant growth of Klebsiella pneumonia resistant to tetracycline, which therefore was discontinued after administration of total dose of 1 75 g within 36 hours. Treatment with colistin 4 mill U/day and cephalothin 4 g/day was started

On January 16 the temperature decreased, but paresis of the left abducens nerve had developed On the next day the temperature rose again, but blood cultures remained negative EEG was normal

On January 18 a new lumbar puncture revealed slightly cloudy spinal fluid under pressure of 490 mm H_2O The WBC was 400/mm³ with 70% mononuclear and 30% polynuclear cells Protein was 99 mg/100 ml and glucose 42 mg/100 ml (blood glucose 118 mg/100 ml) Microscopy and cultures of the spinal fluid revealed no microorganisms

On Jan 20 the EEG was markedly abnormal with

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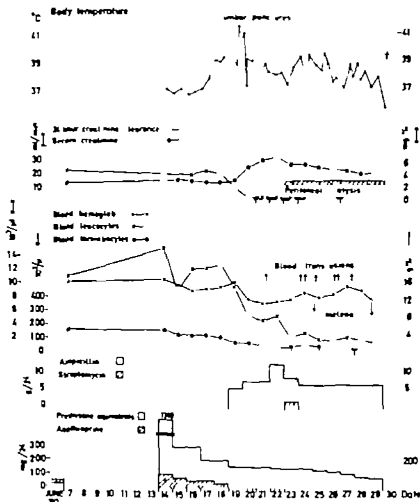


Fig. 2. Temperature, kidney function, Hb, leucocytes, thrombocytes, antibiotic treatment and immunosuppression in case 2.

serum creatinine 3.2 mg/100 ml. Urine cultures were negative. WBC was 15 000/mm³, thrombocytes 154 000/mm³ and Hb 17 g/100 ml. Rejection of the graft was suspected and 1 g methylprednisolone (Solumedrone®) was administered followed on the next day by 300 mg prednisone which was thereafter gradually reduced. Azathioprine, which, due to thrombocytopenia, had been discontinued for a week, was now given dose of 100 mg/day being gradually reduced over the next days. Hereby the kidney function improved little.

On June 18 the temperature rose and at the same time the BP temporarily fell to 105/65 mmHg. Simultaneously the patient got headache and pain in the back. The neck and back were both supple. On June 19 the patient had stiff neck and back.

Lumbar puncture revealed slightly cloudy spinal fluid under pressure of 400 mm H_2O. It contained numerous polynuclear and some mononuclear leucocytes, elevated protein and lowered (<30 mg/100 ml) glucose (unfortunately only determined semiquantitatively). Numerous Listeria monocytogenes were demonstrated by microscopy and later cultures of the spinal fluid. Cultures of the blood grew L. monocytogenes in 7 of 7 glasses.

Treatment with ampicillin, 6 g/day i. was started. However the patient became increasingly confused, stuporous and eventually unconscious, the temperature rose to 41°C, the BP fell temporarily to 105/70 mmHg and the urine production decreased permanently to about 5 ml/min. Hb fell from 16 to 12.1 g/100 ml and to 11.0 g/100 ml on the next 2 days, serum bilirubin rose to 1.3 mg/100 ml and plasma Hb was 6.8 mg/100 ml. The leucocytes and the thrombocytes decreased permanently to very low values in spite of the discontinuation of azathioprine. The bleeding and coagulation times, which were normal on admission, were now >15 min and 15 min, respectively. The prothrombin time was normal and did not increase. Several suggillations and petechiae appeared on the skin. Serum GPT which on admission was 22 U/l rose to 250 U/l serum LDH was now also elevated (unfortunately no isoenzyme determination was done).

On June 22 new lumbar puncture revealed xanthochromatic spinal fluid containing 336 WBC/mm³ with 80% mononuclear and 20% polynuclear cells. Protein

was 234 mg/100 ml and glucose was normal. Some L. monocytogenes were found by microscopy and later in cultures of the spinal fluid. Ampicillin was temporarily increased to 12 g/day and 1 g streptomycin was given.

Due to the reduced kidney function, peritoneal dialysis was started. However, the condition became worse: a grand mal seizure developed followed by cardiac arrest. Both were relieved by relevant treatment. The ventilation now had to be assisted by a respirator. Various cardiac arrhythmias were treated effectively with antiarrhythmic drugs.

During the following days repeated universal epileptic seizures developed, BP fell and increasing pneumonic infiltrations evolved (growth of Klebsiella oxytoca and yeast-like fungi). Relevant treatment was not effective. Urine cultures remained negative. On June 25 the patient had melena and in spite of blood transfusions and other supportive treatment she died on June 30.

At the bacteriologic autopsy numerous Klebsiella oxytoca were cultured from the heart, the lungs, the spleen, and the liver. No Listeria monocytogenes or fungi were cultured. The heart was enlarged. In the posterior wall of the left ventricle a big 10–12-day-old infarction was found. The myocardium was diffusely fibrous. The lungs, specially the lower lobes, contained numerous bronchopneumonic infiltrations. The stomach, the intestine and the colon contained much blood and the mucous membranes were imbibed with blood. In the stomach more acute ulcerations were found. The kidney graft was moderately oedematous and pale, and on the surface two 10–14-day-old infarctions were seen. All anastomoses were sufficient without any reaction. Microscopy revealed chronic vascular rejection. Acute tubular necrosis could not be ruled out because of pronounced autolysis.

The autopsy of the brain revealed a considerable amount of blood in the subarachnoidal space and numerous mas-[illegible]e hematomas measuring up to 4 cm in diameter were [illegible]d in the cerebrum and the cerebellum. No pus was seen [illegible] the arachnoid, and the meninges were not thickened.

[illegible]licroscopically the leptomeninges presented slight patchy perivascular inflammatory reaction and a moderate bleeding beneath the arachnoid. In the white substance marked perivascular edema was present. No microorganisms were demonstrated in Gram-stained sections.

DISCUSSION

Neither of the patients had a history of contact with animals or ill people, nor had they any symptom or sign of gastrointestinal affection, which is supposed to promote the intrusion of Listeria monocytogenes into the organism (1).

Both cases were preceded by an increase in the immunosuppression, which may have activated latent, subclinical infections with L. monocytogenes.

In case 1 stiffness of the neck became apparent 5 days after an increase of the azathioprine dose from 75 to 100 mg/day. The prednisone dose of 30 mg/day was constant throughout the course. No L. monocytogenes were demonstrated in the blood. The bacteriologic diagnosis was established rather late, as a concomitant urinary tract infection was treated for 36 hours with tetracycline, which effectively suppresses the growth of L. monocytogenes. Not until tetracycline had been discontinued for 6 days was it possible to demonstrate L. monocytogenes in cultures of the spinal fluid. Resumed treatment with tetracycline was fully effective.

In case 2 stiffness of the neck appeared 5 days after a massive increase in the steroid dose and administration of a moderate azathioprine dose (Fig. 2) in an attempt to revert a rejection of the kidney graft.

This case was complicated with Listeria sepsis with hemolysis and thrombocytopenia. The latter seems to be due mainly to the depressive action of azathioprine on the bone marrow. Consumption coagulopathy, which, together with hemolysis, has been reported in Listeria sepsis (10), cannot have been present to a significant extent, as the prothrombin time did not increase.

The L. monocytogenes grown were fully sensitive to ampicillin, which is reported to be the drug of choice (4). In spite of the i.v. administration of this drug in high doses, the spinal fluid, after 3 days of treatment, still contained viable L. monocytogenes. Similarly, in a 25-year-old kidney-transplanted woman, in New York, with Listeria meningitis treated with i.v. ampicillin, the spinal fluid still grew L. monocytogenes after 3 days of treatment. However, after 3 weeks of treatment with ampicillin the patient was cured and the spinal fluid had normalized (7).

New in vitro investigations have shown that penicillin or ampicillin in combination with kanamycin or gentamicin kill L. monocytogenes earlier and more effectively than any of the agents alone (3).

The antibiotic treatment given in case 2 seems, however, to have been effective against L. monocytogenes as this microorganism was not demonstrated at autopsy. The characteristic feature of this case was the massive hemorrhages due to the pronounced thrombocytopenia. The meningeal changes were modest and the tissue reaction sparse.

The sudden decrease in kidney function appeared during high fever when the meningeal symptoms evolved and the BP fell; the myocardial infarction must have developed at about this time, when the hemolysis was also demonstrated. These factors seem to have been responsible for the acute renal

failure whereas acute rejection does not seem to have been present.

From experiments with mice it is known that corticosteroids given at the beginning of the infection with L. monocytogenes in a dose corresponding to that given in case 2, greatly suppress the production of immunologically committed lymphocytes by depletion of lymphocytes in lymphoid tissue and by inhibition of DNA synthesis in lymphocytes (8). This leads to diminished proliferation and accumulation of macrophages at infective foci in the tissues (8).

At the same time there is an enhanced accumulation at the infective foci of polymorphonuclear leucocytes, which cannot neutralize L. monocytogenes. This probably represents an ineffective attempt by the host to compensate for the deficit of macrophages at the infective foci (8).

In this context it is emphasized that the first spinal fluid of case 2, who received a massive steroid dose revealed polynuclear pleiocytosis. In case 1 who received a comparatively small steroid dose, the spinal fluid was mononuclear pleiocytotic. So it is possible that the pleiocytosis other things being equal becomes increasingly polynuclear with increasing steroid immunosuppression.

CONCLUSION

The necessary immunosuppression after renal allotransplantation implies an increased risk of clinical listeriosis. One must therefore be prepared to meet cases of Listeria meningitis among kidney transplanted persons specially when the immunosuppression has just been increased. The diagnosis should be made rapidly and as soon as cerebrospinal fluid and peripheral blood have been obtained for culture antibiotic treatment should be started at once. In view of the seriousness of the infection, it seems justified to increase the resistance of the patient by temporarily discontinuing azathioprine and other cytotoxic drugs and reducing the steroid dose at the height of the infection. This does not necessarily involve rejection of the transplant. The kidney function should however be observed carefully.

ADDENDUM

Since the preparation of this manuscript we have had two more cases of listeria meningitis among kidney transplanted patients. They appeared shortly after (5 and 11 days, respectively) intensified immunosuppression with methylprednisolone in high doses (1 ½ and 3 g given over 2 and 3 days, respectively) carried out to revert rejection of the kidney grafts. In both cases polynuclear pleiocytosis was demonstrated in the spinal fluid. Azathioprine was discontinued temporarily and prednisone was reduced in both patients who recovered after treatment with ampicillin plus gentamicin.

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Les prochaines Journées Internationales de Cardiologie de Paris auront lieu à la Faculté de Médecine Pitié Salpêtrière sous la direction des Professeurs J Facquet et J J Welti les 12, 13 et 14 Mai 1975

Renseignements et Inscriptions Professeur J J Welti Hôpital Fernand-Widal 200 fg Saint Denis F 750 10 Paris France

XXIVe Congrès de l'Association des Pédiatres Paris France 8 9 et 10 Juillet 1975 Nouvelle Faculté de Médecine 45 rue des Saints-Pères, F 75006 Paris *Secrétaires généraux* Professeurs A A Hennequet et B Leveque *Secrétariat du Congrès* Expansion Scientifique Française 15 rue Saint-Benoit F 75278 Paris Cedex 06 France.

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RESECTION OF LEFT VENTRICULAR ANEURYSM—LATE RESULTS

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Abstract A survey of 22 patients operated on with left ventricular (LV) infarctectomy during 1967–72 is given Clinical, haemodynamic and angiographic results are discussed. In most patients, in whom pre- and postoperative examination was possible there was improvement concerning anginal pain, dyspnoea and attacks of ventricular tachycardia. Exercise studies revealed a lower heart rate at follow-up I general heart size had decreased. Angiographically there was decrease in end-diastolic and end-systolic heart volume postoperatively with an increased LV ejection fraction.

In some patients with ischemic heart disease (IHD) left ventricular (LV) function can be improved by surgical intervention aimed at either increasing the oxygen supply such as revascularization procedures or decreasing the oxygen demand by reducing the LV diameter The latter approach involves the resection of a postinfarction scar This scar may or may not form a bulge or aneurysm In both cases it may have a similar effect on the function of the LV Although the term infarctectomy would be preferable, the procedure is traditionally called aneurysmectomy The most common indications for this operation are LV failure and angina pectoris other indications being thromboembolism and ventricular arrhythmias resistant to drug treatment.

The aim of this paper is to report the long-term clinical haemodynamic and angiographic results of aneurysmectomy in a group of patients in whom the operation was performed electively: in only one case was the operation combined with a saphenous vein coronary artery bypass.

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MATERIAL

A total of 31 patients have been operated on between 1960 and April 1972. Nine patients were operated on in 1960–66, with hospital mortality of 5 and a late mortality of 3 leaving one survivor Part of this group has been reported earlier (5) Twenty-two patients were operated on after 1967 with a hospital mortality of 4 (18 %) late mortality of 2, and 16 surviving. Two patients still alive 12 and 3 months after operation, are not included since they are being followed up in other hospitals.

Some clinical data on the remaining 14 patients are presented in Table I Only one of the patients was female The mean age was 53 4 years (range 34–65) All patients had suffered at least one myocardial infarction (MI) 2–30 months preoperatively The limiting subjective symptom was angina pectoris and/or effort dyspnoea in 11 cases, ventricular arrhythmias in 2 and general tiredness in one case (no. 9). The patients were classified according to the NYHA classification with modifications (24). All cases but 2 were severely incapacitated.

METHODS

Pre- and postoperative evaluation. This included a 12-lead ECG and when the condition of the patient allowed, a standardized bicycle exercise test in sitting position. Heart rate (HR) and indirect arterial pressure were noted during and at the end of the test, as well as the limiting factor e.g. subjective symptoms, or high HR. In patients of this type we seldom allow the HR to exceed 130 beats/min.

The heart volume (HV) in sitting posture was determined radiologically (19) Angiocardiography was performed either by injecting into the left atrium (LA) through transseptally introduced catheter or directly into the left ventricle (LV) through grey Ödman-Ledin catheter introduced retrogradely via the femoral artery by percutaneous technique Contrast medium 0.3–0.5 ml/kg. (Isopaque® 350 Nyegaard, Oslo) containing 350 mg iodine/ml was injected at rate of 16 ml/sec Simultaneous biplane cine recording of the LV was obtained in right and left anterior oblique projections. Nine-inch image in-

Table I *Patient material*

B=before A=after operation

Case no	Age at op. (y)	Sex	Months from MI to op	Date of infarct ectomy	Follow-up period (mo.)	Symptoms B	Symptoms A	Functional NYHA classification B	Functional NYHA classification A
1	57	♂	6	Dec 1967	58	Angina	Dyspnoea	III b	III a
2	57	♂	5	Jan 1968	57	Angina, dyspnoea	Angina	III b	II–III a
3	60	♂	2	May 1968	52	Angina, dyspnoea	Angina	IV	III a
4	49	♂	9	Jan. 1969	42	Serious dysrhythmias		III b	II
5	61	♂	15	May 1969	39	Dyspnoea, angina		III b	II
6	53	♀	13	Oct 1969	35	Dyspnoea, angina		IV	II
7	65	♂	11	Dec 1969	33	Dyspnoea		III b	II
8	34	♂	13	Oct. 1970	23	Angina	Angina	III b	III a
9	46	♂	8	May 1971	16			II	II
10	55	♂	5	June 1971	15	Dyspnoea, angina	Dyspnoea	III b	II
11	45	♂	29	Oct. 1971	11	Dyspnoea	Dyspnoea	IV	IV
12	54	♂	12	Nov 1971	9	Dyspnoea	Angina	III b	III a
13	54	♂	1962, 1971	March 1972	6	Serious dysrhythmias		IV	II
14	59	♂	18	March 1972	6	Angina		III a	II

tensifiers and 35 mm cameras (Arriflex) running at 50 or 75 frames/sec were used As a rule only one injection was administered to each patient. However in a few cases a second injection was given after changing the patient's posture slightly to show the size of the aneurysm more clearly The cineangiograms were analysed in an optical viewer and the contours of the LV were traced in right anterior oblique projection at end-systole and end-diastole In addition, the size of the aneurysm was measured and expressed in per cent of the total circumference of the LV in diastole The volume of the LV was determined using the method described previously (3). Coronary arteriography was not performed routinely preoperatively and in no case at follow-up

Haemodynamic investigations were carried out in 12 cases. In the other 2 cases only a preoperative LV pressure measurement was performed More complete pre- and postoperative examination was possible in only 6 cases at rest, and in 3 during exercise In 10 cases the postoperative evaluation comprised observations both at rest and during exercise. Cardiac output ($\dot{Q}$) was determined preoperatively using the Fick technique and postoperatively with dye dilution technique (Cardio-Green®). The mean difference (±SEM) between $\dot{Q}$ values according to Fick and dye dilution in this laboratory has been shown to be 0 11±0.08 l/min, when obtained simultaneously (11). All haemodynamic observations were made with the patient in the supine position.

Surgical technique The operative procedure was usually performed through a median sternotomy In a few cases, however a transversal bilateral thoracotomy was performed. Total cardiopulmonary bypass was established using a Kay-Cross disc oxygenator up to 1970 After 1970 an AGA disc oxygenator was used Moderate hypothermia was induced In most cases the aneurysm was adherent to the pericardium During bypass the heart was dissected free as carefully as possible in order to prevent embolization. Furthermore to prevent this risk the aorta was cross-clamped immediately before the aneurysm was incised Most of the aneurysm, including the thrombotic mass usually attached to the wall was removed A small rim was left in order to facilitate closure A row of interrupted heavy silk sutures reinforced by two teflon strips was used for closure followed by a continuous row of silk. The LV was vented during rewarming When necessary the heart was defibrillated The heart was decannulated and the thoracic incision closed leaving chest tubes for drainage for the first 24–48 hours postoperatively

In case 11 the preoperative examination had revealed small mitral insufficiency At operation the papillary muscles, the chordae tendineae and the leaflets seemed to be quite normal The mitral insufficiency was deemed to be a relative one caused by the dilated LV and on this account the mitral valve was left intact. Preoperative coronary angiography now performed routinely in these patients, had shown a total occlusion of the right coronary artery in patient 14 and a saphenous vein bypass was added to the aneurysmectomy Using the method described by Favaloro (13) the saphenous vein was sewed end-to-side to the ascending aorta and to the right coronary artery respectively

Table II *Electrocardiogram and exercise test*

B=before, A=after operation

Case no.		ECG at rest: Abnormal Q	ST elevation	Inverted T	Ectopic beats	Suggestive of aneurysm	Exercise test: Digitalis	W_{max} (kpm/min)	Min	HR	BP	Interrupted due to	Symptoms	Arrhythmia
1	B		+				+	400	4	130	160	Cautiousness	Angina pectoris	
	A					−								
2	B	+		+	−	−		Not perf						
	A	+	−				−	400	6	108	220/100	Angina pect., dyspnoea	Angina pect., dyspnoea	+
3	B				−	+	+	300	4	134	220/130	ECG reaction bigeminy	−	+
	A	↓	+↓	+		−	+	450	2	138		Angina pect.	Angina pect.	+
4	B	+				−	+	600	6	132	170/95	Cautiousness + HR	−	−
	A	+		+	+	−	+	400	6	90	165/95	Arrhythmia	−	+
5	B	+	+	+				Not perf						
	A	+↓	+↓	+↓				400	6	131	185/100	Cautiousness + HR	Dyspnoea	−
6	B	+	+			+								
	A	↓		+↑				300	3	94	180/90	Fatigue	Fatigue	
7	B						+	100	4	120	115/90	Dyspnoea (cautiousness)	Dyspnoea, fatigue	+
	A	↓	↓	+↑				400	6	130	180/95	Cautiousness + HR	−	
8	B	+						600	3	128	190/100	Dyspnoea, fatigue	Dyspnoea, − angina pect.	
	A	+↓		+↑		−	+	600	6	140	185/90	Angina pect dyspnoea, fatigue	Angina pect dyspnoea fatigue	
9	B					+		1 000						−
	A							700	6	130	180/90	Cautiousness HR	Dyspnoea	−
10	B						−	600	3	144	150/80	Dyspnoea	Dyspnoea + angina pect	−
	A	↓	+↓			+	+	400	5	164	145/80	Dyspnoea + VES	Dyspnoea	
11	B			+			+	Not perf						
	A							Not perf						
12	B							100	6	179	150/100	Cautiousness	Fatigue	+
	A	↓	↓					200	3	128	190/100	Fatigue + angina pect.	Fatigue angina pect.	
13	B							Not perf						
	A			+	+		+	450	6	132	180/90	Angina pect., cautiousness	Angina pect. fatigue	−
14	B		( )					300	6	123	175/95	Angina pect + ES cautiousness	Angina pect	
	A							600	6	136	180/90	Cautiousness HR	Fatigue	−

↓ less abnormal

Table III *Haemodynamic findings*
B = before A = after operation

Case no	Rest HR (beats/min) B	A	$\dot{Q}$ (l/min) B	A	CI (l/min m²) B	A	SV (ml) B	A	PA (mmHg) B	A	PCV/LA (mmHg) B	A	LV B	A
1	67	92	5 1	9.2	2.5	4.8	76	99		21/9				
2	99		5.5		2 7		55		41/19					[illegible]00/1
3		112		6.2		3 1		55				6	220/7	
4		60		5.5		2.4		92				5	100/6	128/1
5	80	64	4.9	3.8	2.9	2.2	61	59	45/23		20*	0	136/23	
6	80												135/10	
7	95	75		2.9		1 7		38				1	120/25	112/3
8	70												105/4	
9	73	79	9 3	8.7	4 3	4 1	127	110	27/11		6	6		
10	84	62	4.9	4 4	2.7	2.4	58	70	32/16		22*	3	105/19	110/8
11	104	116	3.3	3 7	1 6	1.8	32	32	58/36	46/18	34	28	110/43	92/74
1	118	93	2.8	4.3	1.6	2.5	23	47	74/37		29*	11		128/1
13	90	54		4.5		2.8		83				7	86/14	120/9
14	71	95	4.6	4 7	2.5	2.5	65	49	13/6		5	3		120/5

* Measured as PCV pressure

RESULTS

Subjective symptoms (Table I). Twelve patients improved and two were unchanged at follow-up. One of the latter (no 11) was in advanced heart failure at operation and died 11 months after operation Of 12 patients who were in function class IIIb–IV before operation, 11 improved and 1 be e more or less symptom-free. Patient 9 had dif and moderately severe symptoms before ration, probably due to a hyperkinetic state (Table III)

Eight patients complained of angina pectoris before operation and this symptom disappeared in four Also 8 cases had dyspnoea before operation and this persisted in only 4 Two cases were severly incapacitated preoperatively by ventricular tachycardias at follow-up they were symptom-free and one patient had resumed his earlier physically demanding work. In this series the subjective results showed no correlation with the patient s age nor with the length of follow-up Of 11 patients below the age of 60 only one returned to work.

Electrocardiogram (Table II) In 4 cases some degree of intraventricular conduction disturbance appeared postoperatively A pathological Q wave was present preoperatively in 12 cases it was unchanged at reinvestigation in 5 and showed regress in 7 cases. A pathological ST elevation in 11 cases became lower or normal in 8 cases The T waves were as a rule unchanged An aneurysmal ECG pattern i.e a deep and broad Q or QS wave followed by ST elevation, was present in 8 cases (57 %) preoperatively and in 4 at follow-up

Exercise studies (Table II) A postoperative work test was carried out in 12 cases The initial work load was chosen according to the individual s expected capacity The maximal work load (W_{max}) varied between 200 and 700 kpm/min (30–110 W) and 8 patients performed at this load for 6 min. A comparison with the preoperative exercise test was possible in 8 cases and showed no difference in maximal work performed However when the same submaximal loads were compared, there was a significantly lower HR ($p<0.001$) at follow-up Similar subjective symptoms appeared during the tests before and after operation The limiting factor was angina pectoris and/or dyspnoea in 6 cases Arrhythmias appeared in 6 cases but were a limiting factor only in one case In the remaining 5 patients the exercise test was discontinued because of unspecific symptoms such as general fatigue

Haemodynamic findings (Table III). Cardiac index (CI) at rest was below 2 6 l/min m² in 4 of 8 cases before and in 7 of 11 cases after operation A direct comparison was possible in 7 cases showing an increase of at least 20 % in 2 cases and a similar

Exercise

Work load (W)		HR (beats/min)		$\dot{Q}$ (l/min)		CI (l/min m²)		SV (ml)		PA (mmHg)		PCV/LA (mmHg)	
B	A	B	A	B	A	B	A	B	A	B	A	B	A
25	15	91	112	8.8	12.6			97	107		34/18		
	30		132		6.7		3.4		50				54
	40		88		10.0		4.3		113				10
	50		104		6.4		3.8		62				6
	50		128		6.5		3.8		51				31
65	50	93	99	14.8	13.1	6.9	6.2	155	132			7*	9
	30		95		6.1		3.4		64				8
	20		118		6.3				53				35
	50		95		9.9		6.2		104				27
40	50	118	131	8.7	10.3	4.7	5.6	73	79		26/15	15	6

decrease in one case. In case 9 $\dot{Q}$ and stroke volume (SV) were high both before and after operation.

$\dot{Q}$ during supine exercise was measured in 10 cases postoperatively. With the exception of case 3 increases of 20–50% from the resting value were noted the degree being related to the work load. In the three patients, in whom a comparison was possible between pre and postoperative $\dot{Q}$ values during exercise variations could be explained by differences in loads.

The LV filling pressure (LVFP) at rest was measured directly or indirectly in 13 cases before and in 12 cases after operation. LVFP above 13 mmHg was found in 7 cases before and in 2 cases after operation. LVFP during exercise remained normal in 5 cases and rose in 4 cases to values between 27 and 54 mmHg. The highest value 54 mmHg during exercise was found in a 60-year-old man whose $\dot{Q}$ was unchanged during work and at rest.

Radiologic findings (Table IV). The preoperative total HV was increased in most cases and exceeded 1 500 ml in 6 cases. At follow-up most hearts were smaller with a decrease in 9 cases of 50–830 ml. In 3 cases the HV had increased 75–220 ml. The total HV showed no close correlation with the LV end-diastolic volume or with the size of the scar.

The findings at LV angiography are presented i Table IV and Fig. 1. A local akinetic area persisted after operation, but was considerably reduced in most cases. The ejection fraction determined angiocardiographically was low in all but one case preoperatively. There was a negative correlation between the end-diastolic LV volume and the ejection fraction ($r = -0.59$ $p < 0.025$). The end diastolic volume was correlated with LVFP ($r = 0.77$ $p < 0.005$). After operation the end-diastolic and end-systolic volumes decreased in all cases. The ejection fraction was considerably larger at follow-up. No complications were connected with the pre and postoperative examinations.

DISCUSSION

I necropsy studies cardiac aneurysm is defined as a localized out-pouching of the cavity of a cardiac chamber with or without outward bulging of the external surface (23). The frequency of postinfarction aneurysms in necropsy series varies around 15% of cases with MI (1 12, 23). With the ad ent of LV cineangiocardiography it has been demonstrated that myocardial scars with resulting local contraction disturbances are common in cases w th IHD (4 6 10). These local changes have been categorized as akinetic, asyneretic or hypokinetic asynchronous and dyskinetic or with systolic paradoxical expansion (18). In some series akinesis and hypokinesis are more common than dyskinesis

Table IV *Radiologic findings*

B=before A=after operation ED=end-diastolic, ES=end-systolic

Case no	Total HV (ml) B	Total HV (ml) A	LV volumes (ml) ED B	LV volumes (ml) ED A	LV volumes (ml) ES B	LV volumes (ml) ES A	Ejection fraction B	Ejection fraction A	Site of wall dysfunction	Estimated size of wall dysfunction (%)
1	1 180	1 000	–	–	–	–	–	–	Inferior	25
2	1 700	1 510	360	210	270	80	0.25	0.62	Inferior	42
3	930	820	260	160	200	70	0.23	0.56	Inferior	31
4	1 640	1 460	260	180	200	100	0 23	0.44	Inferior	30
5	1 700	1 080	370	140	320	50	0 13	0.64	Anterior and apex	47
6	860	810	175	–	65	–	6.63	–	Anterior	28
7	1 240	800	–	110	–	50	–	0.55	Anterior	40
8	785	860	270	–	170	–	0.37	–	Anterior	25
9	1 170	1 270	170	130	130	60	0.24	0.54	Anterior	25
10	1 780	950	230	190	150	70	0.35	0.63	Anterior	33
11	2 080	2 300	420	–	330	–	0.21	–	Anterior	38
1.	1 620	1 100	350	210	280	100	0.20	0.48	Anterior	48
13	1 400	1 060	250	170	190	90	0.24	0 47	Anterior and apex	57
14	940	890	280	190	180	70	0.35	0.63	Inferior	48

(2, 4 17) Local contraction disorders were found in 27 of 52 cases (52%) 35 of 100 cases (35%) and in 42 of 66 cases (64%) in three reported series of patients with coronary heart disease (CHD) (4 6 17) The ventriculograms were taken with rates of 40 30 and 6–12 frames/sec respectively which may have influenced the results to some extent In a series of patients who had chronic postmyocardial nfarction heart failure the incidence of localized ntraction disturbance was 38 of 50 patients (76%) 2

Thus local contraction disorders are common in chronic IHD The consequences for LV function *depend on the size of the non-contractile area* (18 20). When LV function is severly impaired left heart failure will appear This failure can be clinically manifest at rest or only during exercise or can be latent without subjective symptoms but with haemodynamic or angiographic manifestations at rest or during exercise

There are few data available concerning the incidence of different degrees of LV failure in patients after LV aneurysm resection In the excellent review by Graber et al (15) 16 of 18 surviving cases had a subjective and objective improvement according to clinical findings chest X-ray and work tolerance In only one of these cases could the results be demonstrated by means of pre and post operative haemodynamic examinations In other reports too only a few haemodynamic and angiographic reinvestigations have been described (7). A pre- and postoperative angiographic analysis of LV function was published recently (20) as was a haemodynamic study (16).

In recent years aneurysmectomy is often combined with direct revascularization However even in the future isolated aneurysmectomy will be car ried out in cases with diffuse or severe coronary artery disease For this reason the long-term results of aneurysmectomy with haemodynamic and angiographic studies are of interest.

The prognosis of LV postinfarction aneurysm has *been reported to vary from a 5-year survival figure* of 12% (23) to 27% (9) and 69% (1) The cause of death is usually LV failure or reinfarction. This illustrates that as a rule CHD is progressive Consequently the evaluation of the late results after aneurysmectomy should be limited to cases without reinfarction during the observation period and should be interpreted with reference to LV func tion

Subjective results are difficult to evaluate and classify as they depend on many other factors besides the possible change in LV function e g. coronary artery disease and extracardiac factors such as the patient s ambition and anxiety and attitudes to and possibilities for rehabil tation In our group 12 of 14 patients claimed to be improved Six denied

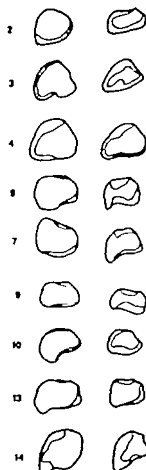


Fig 1 Tracings from cineangiocardiograms of the left ventricle in right anterior oblique projection in 9 patients with LV aneurysm before operation (left) and at follow-up (right). The thick line shows the LV in end-diastole and the thin line in end-systole.

angina or dyspnoea as a limiting symptom in three of them these symptoms were provoked during the exercise test.

Aneurysmectomy has been reported to abolish ventricular tachycardia (8 21 22) In the present material two cases were operated on because of repeated ventricular arrhythmias Postoperatively they were subjectively free from palpitations and the improvement was corroborated by findings at clinical, haemodynamic and angiographic investigations. However in both cases ventricular premature beats appeared during exercise test. Aneurysmectomy has been documented to be of value in isolated cases with severe ventricular arrhythmias but there are no controlled series and the quantitative effect should be studied by longitudinous ECG recording.

Haemodynamic observations were made in 11 cases postoperatively Although 10 cases were improved subjectively the $\dot{Q}$ was low in the majority of cases as found by others (14) However in only a few cases was $\dot{Q}$ determined both before and after operation. According to the postoperative angiographic LV studies the $\dot{Q}$ could have been expected to be higher as practically every case displayed a decrease in end-diastolic and end-systolic volume and an increase in SV and ejection fraction. This discrepancy may be explained by earlier findings that SV according to angiocardiography is larger than with dye dilution (11) This is due to *difficulties in outlining the LV inner contour*

Preoperatively the LV end-diastolic pressure was correlated with the end-diastolic volume. In those cases in whom a comparison was possible this pressure had decreased Postoperatively the end-diastolic pressure was normal in all cases but one and there was no correlation to end-diastolic volume. It has been shown that these parameters have an only limited value in predicting the surgical result (14) Striking correlations between ejection fraction and other parameters of LV function such as end-diastolic volume and stroke work, have been found as well as with the size of the non-contractile area (17 20) The LV function curve was shifted postoperatively towards a normal position (16)

A standardized exercise test is important in the evaluation of the surgical result In this type of patient the exercise test is not intended to determine the maximally tolerated work load which would be potentially dangerous but the load inducing *subjective symptoms or objective signs. In the* absence of such changes the test was discontinued because of the HR attained or cautiousness. Four patients who before operation could not perform an exercise test, tolerated at follow-up a mean load of 65 W Five other cases showed an improvement in the maximally attained work load with lower HR during the test. Only in two cases could the exercise test not be carried out

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IDIOPATHIC HYPERTROPHIC SUBAORTIC STENOSIS

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Abstract. The clinical and laboratory findings in 29 patients with idiopathic hypertrophic subaortic stenosis are presented. Dyspnoea during exercise angina pectoris, syncope combined with left ventricular hypertrophy on ECG and chest X-ray and systolic ejection murmur at the apex and the left sternal border are the most important findings The findings were different in patients below and above 30 years of age. Most of the patients below 30 were in function group I had normal heart volume on chest X-ray and syncope was related to exercise. All patients above 30 had symptoms nearly all were in function groups II–IV and often complained of palpitations, had increased heart volume on chest X-ray sign of enlarged left atrium or atrial fibrillation on ECG. Syncope was not related to exercise, but always associated with palpitation in patient above 35 years of age. Pathologic Q waves were found more often in the younger age group. The differential diagnosis is discussed in relation to fixed aortic stenosis, mitral valve disease, ventricular septal defect, coronary artery disease, and hypertrophic cardiomyopathy without outflow tract obstruction

Idiopathic hypertrophic subaortic stenosis (IHSS) has attracted increasing attention in the recent years mainly for two reasons firstly on account of the peculiar pathophysiology and secondly as an explanation of sudden death in apparently healthy young people (3 12 15).

The haemodynamic findings at rest and after administration of inotropic agents or β-adrenergic blockers have been appropriately defined (2, 6) However the diagnosis based on history clinical findings ECG and chest X-ray is considered to be difficult to establish (7 17).

A correct diagnosis is important because IHSS may be treated with β-adrenergic blockers and/or operation (1, 3, 9, 21, 22, 28) and advice against heavy exercise may be important in the younger age groups IHSS is a rare disease and can easily be overlooked. For these reasons it was decided to make a retrospective study of simple clinical parameters as mentioned above to evaluate which findings are important for the diagnosis of IHSS

PATIENTS AND METHODS

Among 490 patients with aortic stenosis IHSS was diagnosed in 29 They were examined in the Cardiac Laboratory Medical Department B Rigshospitalet Copenhagen, and studied for the first time in the years 1952–72 In two patients the diagnosis was based on autopsy findings and in 27 on haemodynamic findings as described by Braunwald et al (2) The diagnosis was subsequently verified at autopsy in three (26) and at operation in one of the latter 27 patients. A pressure gradient between the left ventricle and aorta at rest, varying between 10 and 165 mmHg, was found in the 27 patients by left heart catheterization In addition to these findings 11 patients had pressure differences of 10–40 mmHg over the right ventricular outflow tract. All records were revised with respect to family history symptoms and sign ECG and PCG findings, chest X-ray and diagnosis on the first admission to the Department. The χ^2-test was used for statistical valuation.

RESULTS

Sex and age Seven patients were females (mean age 37 years, range 9–60) and 22 were males (mean age 31 years range 6–56). Twelve patients were below and 17 above 30 years of age.

Symptoms (Table I) Seven patients had no complaints when seen for the first time in the Department. They were admitted because of the accidental finding of an abnormal ECG or a heart murmur When relating symptoms to age a significantly ($p<0.002$) higher number of patients above 30 years of age were in function groups II–IV had dyspnoea in connection with exercise ($p<0.0?$) and complained of palpitations ($p<0.05$) All patients above

Table 1 *Function groups and symptoms in relation to age in 29 patients with IHSS*

	No. of patients		
	<30 y	≥30 y	Total
NYHA function group			
I	10	3	13
II	2	11	13
III–IV	0	3	3
No symptoms	7	0	7
Syncope	2	4	6
Dizzy spells	2	4	6
Angina pectoris	1	6	7
Precordial oppression	2	7	9
Dyspnoea during exercise	2	13	15
Orthopnoea	0	2	2
Peripheral oedema	1	7	8
Palpitations	3	11	14
Total	1	17	29

30 years of age had symptoms. Two of the three patients in function group I had fainted and the third complained of heart palpitation.

Of 16 patients below 35 years of age six had attacks of syncope and/or dizzy spells appearing in all during or immediately after exercise Of 13 patients of or above 35 years of age six had syncope and/or dizzy spells without relation to exertion, but in all cases there were heart palpitations In four patients syncope or dizzy spells were the only symptoms. Three patients with angina pectoris were treated with nitroglycerin prior to or during the stay in the artment. In one patient the angina vanished after lycerin one felt increasing discomfort and in it was without effect.

Auscultation of the heart A systolic ejection murmur was heard in all patients In 14 patients the maximum of the murmur was at the apex, in 13 at the left sternal border in the 3rd to 4th intercostal space (LSB), and in two the murmur was of the same grade at the apex and at the LSB In nine patients the murmur was heard at the apex the LSB the right sternal border in the 2nd right intercostal space (RSB) and over the neck vessels in ten at the apex the LSB and the RSB in eight both at the apex and the LSB in one only at the apex and in one only at the LSB The murmur never exceeded grade ? of 6 over the neck vessels and at the RSB grade 4 at the apex and at the LSB except in two patients a 6-year-old boy with a grade 6 and a slender 29-year old woman with a grade 5 systolic murmur at the LSB The murmur could vary considerably in intensity in the same patient from one day to another and even sometimes disappear completely A low frequency diastolic murmur and/or a diastolic gallop rhythm was found in 12 patients. On some days it was described as a murmur and on some only as gallop rhythm in the same patient. A mitral snap was not reported in any of the patients but in five patients several observers described a pronounced 1st heart sound at the apex No diastolic murmur was reported at the LSB A thrill was felt in seven patients at the apex, in one at the apex and LSB and in one at the LSB only The ictus was felt in all patients in 28 patients in the 5th intercostal space 1–2 cm lateral to the midclavicular line in one patient in the 6th intercostal space at the midaxillary line.

The BP measured by the cuff method varied from 105 to 140 mmHg systolic and from 50 to 90 mmHg diastolic The pulse pressure ranged from 35 to 60 mmHg, being above 50 mmHg in 20 patients The pulse was quick in 11 patients and five had a pistol shot sound over the femoral arteries

Phonocardiogram (Fig. 1) In 26 patients a PCG was available from the apex and/or the LSB In 22 patients an ejection murmur was demonstrated In four cases no murmur was present on the PCG in these cases the systolic murmur reported at auscultation varied between grades 0 and 3 from one day to another PCG from the apex showed in three patients a 3rd sound in three a 4th sound and in three both a 3rd and a 4th sound In 10 patients in whom a diastolic murmur and/or gallop rhythm was reported at auscultation a PCG was available and in eight of

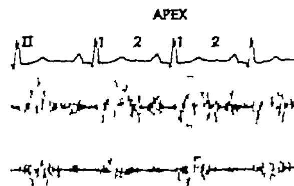


Fig 1 PCG from the apex of a 14-year-old boy with IHSS The ejection murmur and a pronounced diastolic murmur are noticeable. Mingograph 31 B recording 100 and 400 Hz range ECG lead II Paper speed 50 mm/sec.

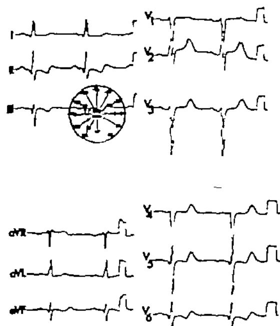


Fig 2. ECG from a 37-year-old woman with IHSS showing left ventricular hypertrophy and septal hypertrophy. Test=1 mV

these patients a 3rd and/or a 4th heart sound was seen on the PCG.

On the PCG from the apex of 18 patients it was possible to measure the interval from the closure of the mitral valve (M) to the start of the systolic murmur and from the end of the systolic murmur to the closure of the aortic valve (A_2). In 16 cases the distance from M to the systolic murmur varied from 0.02 to 0.08 sec and the distance from the systolic murmur to A_2 from 0.02 to 0.08 sec. One patient, in whom the murmur started at M and ended at A_2, had a severe mitral regurgitation shown at angiocardiography and verified at autopsy. In one patient the distance was 0.02 sec from M to the murmur which ended at A_2.

The electrocardiograms were classified according to Goldman (5) modified for children according to Hansen (8). One patient had a normal ECG and two had RBBB. In 21 patients the ECG fulfilled the voltage criteria for left ventricular hypertrophy and in 10 a Q wave suggestive of ventricular septal hypertrophy was present. None of the latter patients had a history of acute myocardial infarction. In five patients the ECG showed combined hypertrophy involving both the septum and the free wall (Fig. 2). Five patients had septal hypertrophy only (Fig 3). Nine of 20 patients of or below 40 years of age and one of nine patients above 40 years had septal hypertrophy ($p<0.08$).

In 23 patients the ECG showed ST segment depression and T wave inversion in V_{4-6}. Eighteen of these patients had left ventricular hypertrophy, four septal hypertrophy as well. Four were treated with digoxine. Two of 12 patients below and seven of 15 patients above 30 years of age had broad notched P waves (>0.12 sec) in lead II as sign of left atrial hypertrophy. Two patients of 56 and 60 years of age had atrial fibrillation. Thus signs of left atrial strain were found in a significantly higher frequency in patients above than below 30 years of age ($p<0.05$). Five of the 11 patients with ECG signs of left atrial strain had gallop rhythm at auscultation and five had dilatation of the left atrium on the chest X-ray.

Chest X-ray. The heart volume was calculated and correlated to body surface area (BSA) from chest X-rays in postero-anterior and lateral projections.

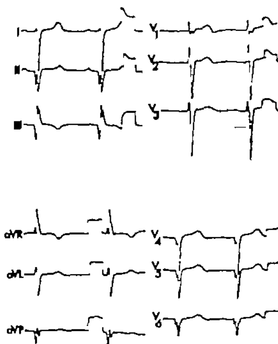


Fig 3. ECG from a 22 year-old man with IHSS showing deep Q_{II-III} and $QS_{V_{1-4}}$ as sign of septal hypertrophy. Test=1 mV

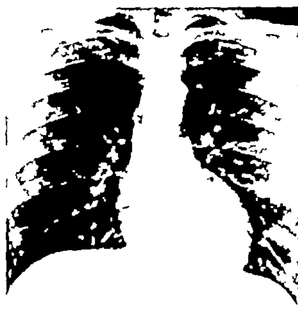


Fig 4 Chest X-ray from a 22 year-old man with IHSS showing *isolated hypertrophy of the left ventricle and normal lung vessels.*

The configuration of the heart and great vessels was evaluated according to Meschan and Farrer Meschan (16). Two patients had normal chest X-rays *The heart volume varied considerably ranging* between 310 and 720 ml/BSA (mean 540) including children. Six patients below and two above 30 years of age had normal relative heart volume <0 05) The shape of the heart showed marked ations from normal to a diffuse enlargement *bly involving all heart chambers.*

n 18 patients bulging of the left heart border etween the apex and the left atrium as a sign of left ventricular hypertrophy was seen (Fig. 4) In six patients the left ventricle was dilated Nine patients had dilatation of the left atrium (Fig. 5) and six had a slight to moderate dilatation of the ascending aorta. *Left ventricular hypertrophy and/or dilatation combined* with dilatation of the left atrium was seen in seven patients one of whom had dilatation of the aorta as well. Five patients had a combination of left ventricular hypertrophy and/or dilatation and dilatation of the ascending aorta. *Five patients had signs of left heart failure One patient showed calcification of* the mitral valve

Family history Three patients had had young siblings who died suddenly and unexpectedly and the father of one patient died in the same way Two patients had siblings *with* non-obstructive cardiomyopathy Two of the 29 patients were siblings. *In eight patients (28 %) a proven or suspect* ed family history of cardiomyopathy was thus found

Other cardiovascular diseases In one patient an atrial septal defect and in another a ventricular septal defect was diagnosed with the platinum electrode *and hydrogen None of the patients had arterial* hypertension when seen in our Department, but a 60-year-old woman had previously been treated for hypertension

Other diseases Chronic lymphatic leucaemia, acromegaly epilepsy and Friedreich s ataxia were diagnosed *in one patient each while two patients were treated for thyrotoxicosis*

Diagnosis on admission was in seven patients mitral stenosis and/or incompetence In one mitral incompetence or ventricular septal defect In four ventricular septal defect In one Adams Stokes at tacks and in one arteriosclerotic heart disease *Congenital heart disease was suggested in seven patients*, and the remaining eight patients were admit ted for evaluation of heart disease None of the patients were admitted to the Department with a correct diagnosis and none on a suspicion of aortic stenosis

DISCUSSION

The findings in our 29 patients are in agreement with other reports (2) The higher mean age of our pa-

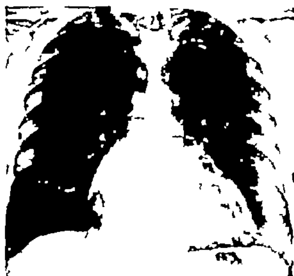


Fig 5 Chest X-ray from 55-year-old man with IHSS *showing dilatation of the left atrium hypertrophy of the left ventricle and normal lung vessels.*

tients compared with that of earlier reported IHSS patients corresponds to the increasing number of IHSS patients above 50 years of age reported by Hardarson et al. (9) and Whiting et al. (27). The most important findings are a history of dyspnoea during or after exercise, attacks of syncope and angina pectoris, at auscultation a systolic ejection murmur with maximum at the apex or the LSB, on ECG left ventricular hypertrophy, and on chest X-ray hypertrophy and dilatation of the left ventricle. In patients with sinus rhythm a double apex beat, pronounced neck vein pulsations, and a sharp arterial pulse are prominent findings (2, 1-, 15).

The physical and laboratory findings differed between the younger and older age groups (27). In the younger age group most of the patients were in function group I, attacks of syncope were related to exercise, on chest X-ray the heart volume was often within normal limits although showing left ventricular hypertrophy (15), and the ECG showed a pathologic Q wave in 50% of the cases. In the older age group all patients had symptoms and most of them were i function groups II–IV with dyspnoea, heart palpitation and attacks of syncope without relation to exercise as the prominent symptoms, increased heart volume on chest X-ray and signs of enlarged left atrium or atrial fibrillation on the ECG.

None of our patients were admitted with a correct diagnosis. This finding accords with that of Whiting et al. (27), who reported that less than 40% of their patients were correctly diagnosed before catheterization. They found the most important differential diagnostic problems to be related to coronary artery disease (CAD), mitral regurgitation, ventricular septal defect and valvular aortic stenosis. This is in agreement with our report except for the valvular aortic stenosis. The clinical findings in fixed aortic stenosis, the systolic ejection murmur with maximum over the basis and projection to the neck vessels, the weak arterial pulse, the low pulse pressure and the dilated ascending aorta on chest X-ray make this disease easily distinguishable from IHSS. Patients with combined IHSS and valvular discrete subvalvular, supravalvular aortic stenosis or calcified aortic val es without stenosis were excluded in our series. The clinical findings in these patients are indistinguishable from pure fixed aortic stenosis and the correct diagnosis depends on haemodynamic and angiocardiographic examinations (11, 19).

A PCG seems to be most helpful in making a correct diagnosis (10, 14). The systolic murmur is of ejection type and a delay from M to the start of the systolic murmur and/or from the end of the murmur to A_2 from 0.02 to 0.08 sec is nearly always present, as demonstrated in our patients and as reported by Lindgren and Epstein (14). This is in contrast to patients with rheumatic mitral regurgitation or other organic disease of the mitral valve, in whom a true holosystolic murmur is always found at the apex. If a holosystolic murmur is heard at the apex in a patient with IHSS, an organic lesion of the mitral valve should be expected, as was the case in one of Lindgren and Epstein s patients and as we have seen in one of our patients at angiocardiography and autopsy.

The findings of enlarged left atrium on chest X-ray combined with a diastolic and a faint systolic murmur (1, 3) and accentuated 1st heart sound (10) at the apex, calcification of the mitral valve and atrial fibrillation as seen in one of our patients and recently reported by Shabetai and Davidson (24) may completely simulate mitral valve disease. Lack of mitral opening snap and demonstration of an ejection murmur at the apex on the PCG should arouse a suspicion of IHSS. In these cases ultrasound echocardiography of the anterior mitral valve and the ventricular septum may give a correct diagnosis (24, 25).

Angina pectoris, a pathologic Q wave, diffusely enlarged heart or prominent left heart border on chest X-ray combined with a systolic murmur at the apex might give a diagnosis of CAD complicated by previous myocardial infarction and mitral regurgitation (4, 20). IHSS and CAD may coexist (7, 13, 17), as was the case in one of our patients with paradox effect of nitroglycerin, who had a complete obstruction of the right coronary artery revealed at coronary arteriography. Signs of left ventricular hypertrophy on ECG, of an ejection murmur at the apex on PCG, of a sharp arterial pulse, and in some patients of paradox effect of nitroglycerin, should lead to a correct diagnosis. The final diagnosis might depend on both haemodynamic nvestigation and selective coronary arteriography.

A ventricular septal defect might be suspected *because of medium to low frequency systolic* murmur with maximum at the LSB. Demonstration *of an ejection murmur on the PCG, normal flow* pattern on chest X-ray, signs of left entricular

hypertrophy which is only seen in ventricular septal defect with large shunts and increased flow pattern on chest X-ray (23) should lead to a correct diagnosis.

Hypertrophic cardiomyopathy without outflow tract obstruction may clinically be indistinguishable from IHSS and the diagnosis can only be established after haemodynamic and angiocardiographic examinations (4 6, 18)

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DIAZEPAM IN CARDIOVERSION

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Abstract Diazepam has been used to an increasing extent in cardioversion since voiding general anaesthesia simplifies the procedure. The present study concerns the effect of diazepam on BP and blood gases in 13 cases of cardioversion. A moderate fall of both systolic and diastolic BP occurred. The arterial pO and pCO_2 did not change significantly

Diazepam has been used to an increasing extent in cardioversion in recent years (1 2, 4 5 6 7 8 9 10) since avoiding general anaesthesia reduces the frequency of complications especially the incidence of premature ventricular beats (4) Using diazepam also simplifies the cardioversion procedure as the skilled help of an anaesthesiologist is not mandatory Diazepam is generally claimed to produce amnesia in nearly all cardioversion patients without causing any troublesome cardiovascular or respiratory depression (2 4 7 8 9 10)

As we could not find any systematic report be hind this general opinion, we started the present study of blood pressure and blood gases in cardioversion with diazepam

MATERIAL AND METHODS

The study concerned 1 patients with 13 cardioversion procedures. Six were males and six females aged 52 74 years. Seven patients had chronic and one acute atrial fibrillation. In three patients the indication for cardioversion was acute and in one chronic atrial flutter One of the 12 patients had valvular heart disease one treated thyreotoxicosis, one cor pulmonale and four arterial hypertension. In five patients no disposing factor was found.

Patients with chronic atrial fibrillation or flutter had effective anticoagulant treatment for 3 weeks before cardioversion, if not contraindicated Digoxin treatment was stopped 3 days before the procedure Quinidine prophylaxis was started 1–2 days before the countershock (quinidine durules 1.2 g/day) in all patient with chronic arrhythmia. The patients had fasted for at least 4 hours.

N premedication was given. The patients breathed air throughout the procedure. Diazepam was administered i. at rate of about 5 mg/2 3 min until the patient became somnolent with slurred speech or fell asleep. The total dose required varied between 5 and 45 mg (mean 23) The ynchronized DC countershocks were delivered through anterior chest and back paddles about 2 min after the last injection. The energy of the first shock was set to 100–300 W (mean 177). In 3 cases second shock of 300 W was needed, which restored sinus rhythm in one case

BP was recorded every second minute from just before the diazepam injection until about 10 min after the countershock. Blood for pO_2 and pCO_2 analysis was taken by puncturing the femoral artery just before the diazepam injection and immediately following the countershock, i 4–6 min after the last injection.

When alert after the procedure, the patients were asked if they recalled anything unpleasant from the treatment

RESULTS

Delivery of the countershock caused transient arousal and usually a groan or a slurred comment, but when alert 15–30 min later all patients denied any unpleasant event.

The BP range was 185–110/110–80 mmHg before the diazepam injection, and the minimum values after injection and cardioversion were 145–100/105–60 mmHg, a significant decrease of both pressures. The maximal systolic drop in each patient showed a range of 0–50 mmHg (mean 19) and the maximal diastolic change of −45–+5 mmHg (mean −14) There was no correlation between the diazepam dose given and the maximal BP fall

The arterial pO range was 69–107 before and 60–108 mmHg after the diazepam injection and cardioversion, a non-significant difference The change was −.5–+14 mmHg (mean −7). The arterial pCO was 30–44 before and 30–47 mmHg after

the diazepam injection and cardioversion a non-significant difference The change varied between −_ and +3 mmHg (mean 0)

DISCUSSION

The diazepam doses needed to produce somnolence/sleep and amnesia vary considerably in the present series between 5 and 45 mg i.v In our experience elderly patients with congestive heart failure need the smallest doses and alcoholic patients the largest. A high requirement in alcoholic patients has also been reported by others (1 7) The upper dose limit varies in the literature between _5 and 80 mg i v (1 7) and was in the present series 45 mg i v If a reasonable dose of diazepam proves ineffective it has been suggested that i v morphine sulphate should be added (1)

In the present series amnesia was induced in all patients In 3 pooled series totalling 74 patients the incidence of non-amnesia was 5% (4 7 10) The present study confirms the general statement of many reports that diazepam in cardioversion does not cause any troublesome cardiovascular or respiratory depression (2 4 7 8 9 10)

However the systolic BP dropped maximally 50 mmHg and the diastolic BP 45 mmHg (mean maximal fall 19 and 14 mmHg, respectively) A decline of this magnitude could theoretically be critical for the occasional patient but may also occur in general esthesia. A mean pressure fall of the same nitude as in the present study occurred during uction of anaesthesia in patients premedicated for cardiac surgery whether barbiturates or diazepam (1 -30 mg) were used (3) In that study the maximal BP effect was not reached until 6 min after the diazepam injection. In the present study only 2 min had elapsed when the countershock was elicited so the BP changes reported here probably represent the net effect of the injection plus the countershock. However the immediate object of the present study was the cardioversion procedure as performed clinically

The pO changes were usually minor but the decrease in one patient without acute or chronic respiratory disease from 85 to 60 mmHg invites caution especially in patients with respiratory failure. In one series oxygen 5 l/min was administered routinely throughout the procedure (7) Equipment for manual ventilation should anyhow be available as occasional cases of transient apnoea have been reported though they did not require assisted ventilation (8)

We have now been using diazepam in all cardioversions for 2 years and the only complications noted are that the amnesia induced has been inadequate in 2 of about 100 patients

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INVOLUTION OF POLYCYSTIC KIDNEYS DURING ACTIVE TREATMENT OF TERMINAL UREMIA

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Abstract. Following institution of chronic dialysis and/or renal transplantation for terminal uremia, four consecutive patients observed for 15–48 months with their polycystic kidneys in situ invariably showed a rapid relief of symptoms (pain, hematuria) followed by a gradual but steady involution of the kidneys. The mechanism and the practical clinical implications are discussed.

a gradual reduction in kidney size was in evidence. The longer the period of observation, the more pronounced was the observed involution of the polycystic kidneys. The significance of this finding is discussed from the pathogenetic and clinical points of view.

It is well established that polycystic kidneys usually are considerably enlarged before marked reduction in renal function is in evidence (1). Although the rate of growth of the polycystic kidneys in relation to the progression of the disease has not been studied in detail, it appears likely that the increment in kidney size decreases during progressive renal insufficiency until the stage of terminal renal failure is reached.

Among many other observations of pathophysiological and clinical significance, the advent of chronic hemodialysis and renal transplantation has made it possible to observe the development of polycystic kidney disease beyond the stage at which the patients previously died in terminal uremia. None of the larger materials on dialysis and transplantation in patients with polycystic kidneys in situ do, however, specify this problem (2, 4, 5). While perusing the literature in preparation for the present paper, we found only one publication illustrating a marked reduction in the size of the polycystic kidneys of a 37-year-old man two years after successful grafting (3).

The present paper supports and extends this observation. In four consecutive patients who had their polycystic kidneys in situ for more than 15 months after institution of active treatment of terminal uremia by dialysis and/or transplantation

PATIENT MATERIAL

In the period Jan. 1965–June 1974, 14 patients with terminal uremia due to polycystic kidney disease were treated at Rigshospitalet.

Nephrectomized patients. One patient was totally nephrectomized during dialysis and 4 were totally nephrectomized in connection with necrokidney transplantation. Four of the 5 patients were from our early experience in 1965–1969, when bilateral nephrectomy was carried out on liberal indications in the majority of all patients actively treated for terminal uremia.

Non-nephrectomized patients. In 9 patients, the polycystic kidneys were left in situ. Five of them, 2 on chronic dialysis, 2 back on chronic dialysis after rejection of necrokidney grafts and 1 recently transplanted with necrokidney, have been followed for less than 6 months on active treatment. In these cases, possible changes in the size of the polycystic kidneys have not yet been studied due to the short observation period.

Four patients, all transplanted with necrokidneys, have been observed for periods exceeding 15 months (range 15–48) and in these patients a study of the clinical course and of changes in kidney size has been performed. Their case histories are presented below.

CASE HISTORIES

Patient 1, woman, born in 1930 (TP 63).

Family history. Marked disposition for polycystic kidney disease on paternal side; sister suffering from polycystic kidneys, died from subarachnoideal hemorrhage in 1973.

Course of illness. In 1959 acute pyelonephritis followed by attacks of flank pain and macroscopic hematuria

at monthly to bimonthly intervals. In Aug 1959 left-sided lumbotomy with puncture of cysts without evident relief. In Dec. 1959 surgical drainage of right-sided perirenal abscess. Rising serum creatinine since 1962 In terminal renal failure in the spring of 1970 still having frequent attacks of recurrent flank pain and hematuria.

Diagnostic verification I. urography in 1959 inspection of kidneys at surgery in 1959 renal angiograms in March 1970 and May 1974

Kidney and liver size before transplantation Both kidneys considerably enlarged from 1959 to 1970 No liver enlargement.

Transplantation was carried out without antecedent dialysis with a necrokidney on May 21 1970 Immediate onset of graft function

Course of disease after transplantation No episodes of flank pain or hematuria after transplantation. Kidney size gradually decreasing. Kidneys no longer palpable from Sept. 71 16 months after grafting

Present status (June 74 49 months after transplantation) Excellent clinical condition. Revalidated to housework plus part-time work as secretary Kidneys not palpable No liver enlargement. Serum creatinine 0.09 mmoles/l 24-hour endogenous creatinine clearance 80–90 ml/min BP 115/70 Urine less than 100 mg protein/24 hours, sterile on culture. Medication. azathioprine 100 mg and prednisone 2.5 mg/day

Patient 2 man, born in 1919 (TP 124)

Family history N known cases of polycystic kidney disease in the family

Course of disease No definite urologic symptoms. In March 1971 examined by own doctor due to uremic symptoms. Transferred to Rigshospitalet with creatinine clearance of 8 ml/min.

Diagnostic verification Renal angiograms in April 1971 and I Oct. 1972. Autopsy in Dec 1972

Kidney and liver size before dialysis and transplantation Neither kidneys nor liver were palpable, possibly due to marked obesity

Dialysis and transplantation Regular dialysis in July–Nov 71 Transplanted on Nov 2, 1971 with necrokidney Immediate function which remained good until July 1972 when chronic rejection was diagnosed clinically and by graft biopsy Died in uremia with terminal septicemia on Dec. 27 1972 Autopsy revealed few 1 cm large cysts in the liver and typical polycystic kidneys measuring 16×10×10 cm each.

Course of disease during dialysis and after transplantation. As before active treatment, no local urologic symptoms Kidneys not palpable, liver not palpable.

Patient 3 man born in 1921 (TP 133)

Family history Marked disposition to polycystic kidney disease on maternal side One older brother died from uremia at the age of 40. A twin brother suffering from polycystic kidney disease was transplanted at another hospital in 1973

Course of disease Between 1950 and renal transplantation in 1971 numerous episodes of flank pain and macroscopic hematuria. *Rising serum creatinine since* 1964 In terminal renal failure in 1971

Diagnostic verification I v urography in 1964 Renal angiograms in Oct. 1970 and March 1974

Kidney and liver size before transplantation and dialysis Kidneys large and readily palpable between 1964 and 1971 No liver enlargement.

Transplantations and dialysis Transplanted without antecedent dialysis with necrokidney on Dec. 3 1971 *Acute rejection followed by graftectomy on Dec. 9 1971* On regular hemodialysis between Dec. 9 1971 and Feb 2, 1973 when retransplanted with a necrokidney which is still functioning.

Course of disease on dialysis and after successful transplantation Since admission to the regular hemodialysis program following failure of first graft there have been no episodes of flank pain or hematuria. Polycystic kidneys, gradually decreased in size were no longer palpable in July 1972, 7 months after start of hemodialytic treatment.

Present status (June 1974 30 months after institution of dialysis and 16 months after successful transplantation) Clinically in good condition, works 6 hours per day in previous job as accountant. Kidneys not palpable. Liver 4–5 cm below right costal margin (It is not possible to state from the records when the liver which was not palpable before dialysis started to increase in size.) Serum creatinine 0.10–0.11 mmoles/l 24-hour endogenous creatinine clearance 60–65 ml/ml BP 140–150/90–100 Urine less than 150 mg protein/24 hours and sterile on culture Medication: azathioprine 125 mg and prednisone 15 mg/day

Patient 4 woman born in 1914 (TP 135)

Family history Not conclusive.

Course of disease Slight uremic symptoms since 1966. N flank pains or hematuria. In terminal uremia in June 1971

Diagnostic verification I v urography in 1966, renal angiograms in May 1971 and March 1974

Kidney and liver size before dialysis and transplantation. Kidneys palpable and slowly growing since 1966. Liver not palpable.

Dialysis and transplantation. On regular dialysis between June 1971 and Dec 1 1971 when transplantation with a necrokidney which is still functioning was performed

Course of disease on dialysis and after transplantation. As before, no flank pain no hematuria, Kidneys very large and readily palpable at transplantation in Dec. 1971. Kidneys still palpable in Jan 1973 no longer palpable in Aug. 1973 26 months after start of dialysis and 20 months after grafting.

Present status (June 74 36 months after institution of dialysis and 30 months after successful transplantation). General condition good Receives pension but manages full-time housework. Kidneys not palpable, liver not enlarged. Serum creatinine 0 09 mmoles/l, 24-hour endogenous creatinine clearance 60–70 ml/min, BP 140–160/90–100 Urine less than 100 mg protein/day Intermittent asymptomatic bacteriuria. Graft urogram normal. Medication: azathioprine 100 mg and prednisone 5 mg/day

ANGIOGRAPHIC TECHNIQUE

All patients with terminal renal failure are examined with renal angiograms and arteriography of the iliac vessels as part of our "pretransplantation survey". Routine ultrasonic scanning was not carried out and i.v. urography even with high doses of contrast and nephrotomography does not reveal the kidney area in polycystic kidneys with very poor function. Therefore, apart from physical examination, the only possibility of reexamining the patients with the aim of demonstrating a change in kidney size was to repeat the renal angiograms.

Like urography, renal angiography does not give a sharp delineation of the kidney surface in polycystic kidneys with exceedingly low function. It was therefore necessary to search for the most peripheral arterial ramifications in the right and left kidneys which should be verified as anatomically identical in the first and the second renal angiogram of each patient. The ramification points were connected by straight lines, and the area of the resulting polyeders was measured by planimetry (Fig. 1). The percentage reduction in the size of the polyeders was taken to represent the percentage reduction in total kidney size.

Possible errors caused by slight variations in the film-focus distance were corrected for by planimetry of the area of the same corpora vertebralis in both examinations. This correction only proved to be of importance in one case, in whom the first and second angiograms were carried out by slightly different techniques.

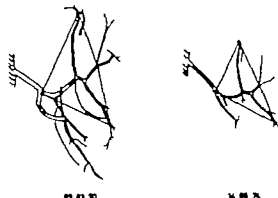


Fig. 1. The most peripheral arterial ramifications which could be shown to be identical in two arteriograms on patient 1 taken with an interval of 30 month.

RESULTS

Clinical symptoms and signs. As illustrated by the case histories, hematuria and flank pain subsided rapidly following grafting (pat. 1) as well as after institution of regular dialysis (pat. 3). In the three patients in whom the kidneys were clinically palpable (pats. 1, 3 and 4) they gradually diminished in size and were no longer palpable 16 months after grafting (pat. 1), 7 months after institution of regular dialysis (pat. 3) and 20 months after grafting (pat. 4).

Thus clinical symptoms apparently vanish long before reduction in kidney size is in evidence. The results are summarized in Table I.

Kidney size. As described above, an attempt was made to estimate the percentage reduction in kidney size by renal angiography. Table II shows that a reduction was found in all four patients and that it was roughly parallel to the active treatment period.

DISCUSSION

The present material of 4 consecutive patients with their polycystic kidneys in situ for more than 15 months after institution of chronic hemodialysis and/or renal transplantation demonstrates that the kidneys invariably undergo a gradual involution and that the degree of shrinkage is roughly proportional to the observation period. This supports and extends the only other published observation of a similar phenomenon in a male patient, who was

Table I. *Clinical data of the four patients*

Pat. no.	Sex	Born in		Pain	Hematuria	Infection	Palpable kidney	Palpable liver
1	♀	1930	Before TP	+++	++	+	++	0
			After TP	0	0	0	0	0
2	♂	1919	Before dial. and TP	0	0	0	0	0
			After dial. and TP	0	0	0	0	0
3	♂	1921	Before dial. and TP	+++	+++	( )	+++	0
			After dial. and TP	0	0	0	0	++
4	♀	1914	Before dial. and TP	0	0	0	+++	0
			After dial. and TP	0	0	0	0	0

at monthly to bimonthly intervals. In Aug. 1959 left-sided lumbotomy with puncture of cysts without evident relief In Dec 1959 surgical drainage of right-sided perirenal abscess. Rising serum creatinine since 1962. In terminal renal failure in the spring of 1970 still having frequent attacks of recurrent flank pain and hematuria.

Diagnostic verification I urography in 1959 inspection of kidneys at surgery in 1959 renal angiograms in March 1970 and May 1974

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Transplantation was carried out without antecedent dialysis with a necrokidney on May 21 1970 Immediate onset of graft function.

Course of diseas after transplantation No episodes of flank pain or hematuria after transplantation. Kidney size gradually decreasing Kidneys no longer palpable from Sept. 71 16 months after grafting.

Present status (June 74 49 months after transplantation). Excellent clinical condition. Revalidated to housework plus part-time work as secretary Kidneys not palpable. No liver enlargement. Serum creatinine 0.09 mmoles/l 24-hour endogenous creatinine clearance 80–90 ml/min BP 115/70 Urine less than 100 mg protein/24 hours, sterile on culture. Medication. azathioprine 100 mg and prednisone 2.5 mg/day

Patient 2 man, born in 1919 (TP 124)

Family history N known cases of polycystic kidney disease in the family

Course of diseas No definite urologic symptoms In March 1971 examined by own doctor due to uremic symptoms. Transferred to Rigshospitalet with creatinine clearance of 8 ml/min.

Diagnostic verification Renal angiograms in April 1971 and in Oct. 1972. Autopsy in Dec. 1972.

Kidney and liver size befor dialysis and transplanta- Neither kidneys nor liver were palpable, possibly to marked obesity

Dialysis and transplantation Regular dialysis in ly–Nov 71 Transplanted on Nov 2 1971 with necrokidney Immediate function, which remained good ntil July 1972, when chronic rejection was diagnosed clinically and by graft biopsy Died in uremia with terminal septicemia on Dec. 27 1972 Autopsy revealed few 1 cm large cysts in the liver and typical polycystic kidneys measuring 16×10×10 cm each

Course of disease during dialysis and after transplantation As before active treatment, no local urologic symptoms. Kidneys not palpable, liver not palpable

Patient 3 man, born in 19?1 (TP 133)

Family history Marked disposition to polycystic kidney disease on maternal side. One older brother died from uremia at the age of 40 A twi brother suffering from polycystic kidney disease was transplanted at another hospital in 1973

Course of disease Between 1950 and renal transplantation in 1971 numerous episodes of flank pain and macroscopic hematuria. Rising serum creatinine since 1964 In terminal renal failure in 1971

Diagnostic verification I v rography in 1964 Renangiograms in Oct. 1970 and March 1974

Kidney and liver size before transplantation dialysis Kidneys large and readily palpable between and 1971 No liver enlargement

Transplantations and dialysis Transplanted kt antecedent dialysis with necrokidney on Dec. 3, 197 Acute rejection, followed by graftectomy on Dec. 9 197 On regular hemodialysis between Dec. 9 1971 and Feb 1973 when retransplanted with a necrokidney which still functioning.

Course of disease on dialysis and after success transplantation. Since admission to the regular hen dialysis program following failure of first graft have been no episodes of flank pain or hematuri Polycystic kidneys gradually decreased in size were nc longer palpable in July 1972, 7 months after start hemodialytic treatment.

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Patient 4 woman, born in 1914 (TP 135)

Family history Not conclusive.

Course of disease Slight uremic symptoms since 1966 No flank pains or hematuria. In terminal uremia in Ju 1971

Diagnostic verification I v urography n 1966, angiograms in May 1971 and March 1974

Kidney and liver size before dialysis and transp tion Kidneys palpable and slowly growing since 1966 Liver not palpable

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Present status (June 74 36 months after institution of dialysis and 30 month after successful transplantation) General condition good Receives pension, but manages full-time housework. Kidneys not palpable liver not enlarged. Serum creatinine 0 09 mmoles/l, 24-hour endogenous creatinine clearance 60–70 ml/min BP 140–160/ 90–100 Urine less than 100 mg protein/day Intermittent asymptomatic bacteriuria. Graft urogram normal. Medication: azathioprine 100 mg and prednisone 5 mg/day

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RENAL EXCRETION OF VANCOMYCIN IN KIDNEY DISEASE

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Abstract The renal elimination of vancomycin has been determined in 18 patients. In 4 anuric patients in intermittent haemodialysis the dosage of vancomycin necessary to treat infections with penicillin-resistant strains of Staphylococcus aureus was determined. In 14 patients with varying degrees of renal insufficiency vancomycin, creatinine and [125]iothalamate clearances were measured and found to be closely correlated. After administration of the initial vancomycin dose and attainment of the serum concentration desired, the maintenance dose can be calculated on the basis of the GFR.

In patients with renal insufficiency and in kidney transplant recipients infections with Staphylococcus aureus and Gram-negative rods are a very common complication (5 12). These bacteria are often resistant to commonly used antibiotics and therefore potentially ototoxic and nephrotoxic antibiotics must be employed such as streptomycin kanamycin, gentamycin and vancomycin. Ototoxicity appears to be particularly common in patients with renal insufficiency since the elimination of these substances is primarily by way of the kidneys (3 4 6 15). Treatment of renal-insufficient patients with these substances necessitates monitoring of the serum concentration.

The purpose of the present study was to investigate whether vancomycin administration could be calculated on the basis of renal function. We therefore determined the renal excretion rate for vancomycin in patients with varying degrees of renal insufficiency.

MATERIAL

Eighteen patients, 8 women and 10 men aged 1–68 years, were studied. These patients were divided into 2 groups.

Group I consisted of 4 patients, 3 women and 1 man, aged 21 37 years. These patients were anuric and in chronic intermittent haemodialysis. Bilateral nephrectomy had been performed in all and one had a nonfunctioning renal graft. Body weight varied from 43 to 60 kg. Serum vancomycin was measured during treatment of infections caused by Staphylococcus aureus (Fig. 1).

Group II consisted of 14 patients with serum creatinine values between 0.8 and 19.3 mg/100 ml. [125]Iothalamate clearance was 60–100 ml/min in 3 patients, 20–60 in 7 and less than 20 ml/min in 4. Other data are given in Table I.

METHODS

A cuprophane membrane, 1 m² 11 μm thick, was used during dialysis which varied from 8 to 10 hours.

In patients in group II vancomycin, creatinine and [125]iothalamate clearance (C_{Vanc}, C_{Cr}, C_{Io}) determinations were made and the clearance ratios C_{Vanc}/C_{Io} and C_{Vanc}/C_{Cr} were calculated. A classical clearance technique was employed (17).

[125]Iothalamate was supplied by Amersham Radiochemical Centre England. Total dose was 10–25 μCi.

Vancomycin-hydrochloride. Measurement of vancomycin in serum and urine was done by an agar diffusion method (7). Dishes, 287 287 mm, were employed with Difco antibiotic medium no II as basis layer and Difco antibiotic medium no V as growth layer with Staphylococcus aureus PSTEL 19328 embedded as indicator organism. Sixty-four cylindrical holes were punched into the agar dish so that standard dilutions of vancomycin and all samples from a single patient could be applied to the same dish.

Using a stock solution of vancomycin-hydrochloride dissolved in phosphate buffer (pH 6.2) standard dilution of vancomycin with human serum was made with concentrations of 100 80, 40 20 and 10 μg/ml. Diameters of the inhibition zones produced by these dilutions were used to plot a dose-response curve.

Zones of inhibition were measured for both undiluted and diluted (1 2) serum samples (dilution medium human serum) and for urine undiluted and diluted 1 2 or diluted 1 and 1 5 dependent upon the expected concentration (dilution medium. 0.9% NaCl). Three to four zones of inhibition were read for each dilution and the

Table I *Kidney function studies and excretion of vancomycin-hydrochloride in group II*

CG = chronic glomerulonephritis, CP = chronic pyelonephritis, RAT = renal allotransplantation

Pat. no	Sex	Age (y)	Diagnosis	Proteinuria (g/24 h)	Serum albumin (g/100 ml)	Clearances (ml/min) C_{In}	C_{Cr}	C_{Vanc}	Clearance ratios C_{Vanc}/C_{In}	C_{Vanc}/C_{Cr}
1	♂	48	CG	3 6	2.9	2.6	3.5	2.4	0.92	0.69
2	♂	28	RAT	1 1	[illegible]9	9.2	16	7.4	0.81	0 46
3	♀	44	CP	1.6	2.9	10	17	8.3	0.80	0 49
4	♂	62	Analgesic nephropathy	0.8	2.9	19	28	16	0.84	0.57
5	♀	43	RAT	0	2.4	30	52	28	0.95	0.54
6	♀	34	CP	0.8	2.8	30	53	20	0 67	0.38
7	♂	25	RAT	0.6	2.0	40	61	32	0.80	0.52
8	♂	50	Nephrocalcinosis	0	3 4	51	86	38	0 74	0.44
9	♀	22	RAT	0	2.4	54	68	38	0 70	0 56
10	♀	68	No kidney disease	0	2.8	56	85	42	0 75	0 49
11	♂	21	RAT	0	2.8	58	72	37	0.64	0.51
12	♂	55	N kidney disease	0	3 4	67	89	48	0 72	0.54
13	♂	28	RAT	0	3.0	72	119	48	0.67	0.40
14	♂	45	CG	13.5	1.3	103	123	101	0.98	0 82
Average		41			2.7	43	62	33	0.79	0.53
S.D					0.6	28	37	24	0 11	0 11
S.E.M.					0.2	7.5	9 9	6.6	0.03	0 03

average zone diameter was used in plotting the curve Each serum sample was represented by the result of 7–8 determinations. Concentration of serum vancomycin varied from 5 6 to 29 μg/ml (mean 18 4±5.5).

RESULTS

...up I In four anuric patients serum concen...ons of vancomycin were determined during ...c use of the agent (Fig 1) As shown in the Figure the administration of 1 g vancomyci... per week was sufficient to maintain serum ...centration. This dose corresponds to 7% of ...mal dose On the basis of the quite ... serum concentrations between doses it can ... concluded that only small amounts of v... transversed the dialysis membrane

Group II In 14 patients (Table I Figs ... and 3) with ...iothalamate clearances var...

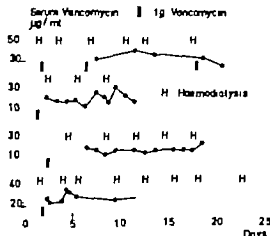


Fig 1 Variations of serum vancomycin concentrations in patients in group I.

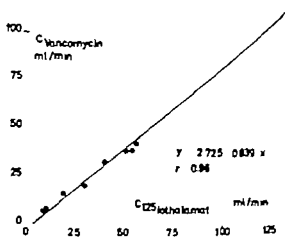


Fig 2 Vancomycin clearance compared to ...iothalamate clearance in group II

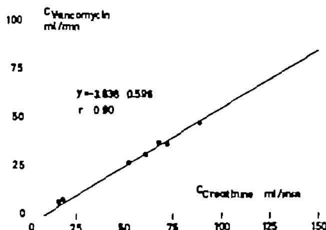


Fig. 3. Vancomycin clearance compared to creatinine clearance in group II.

from 2.6 to 103 ml/min and creatinine clearances from 3.5 to 123 ml/min a good correlation was found between C_{In} and C_{Vanc} ($p<0.001$ re gression analysis and -values given in Fig. 2). A good correlation was also found between C_{Vanc} and C_{Cr} ($p<0.001$ regression analysis and r values given in Fig. 3). The C_{Vanc}/C_{In} ratio was calculated to be 0.79 ± 0.11 and C_{Vanc}/C_{Cr} ratio 0.53 ± 0.11. Clearance ratios were independent of the degree of renal insufficiency. There was a negative limit correlation between C_{Vanc}/C_{In} and serum albumin concentrations ($0.05<p<0.1$). In a patient with the nephrotic syndrome (no. 14) the C_{Vanc}/C_{In} ratio was 0.98.

DISCUSSION

The chemical structure of vancomycin is not known but its molecular weight has been found to be approximately 1 800 (13). This substance can form complexes in vitro with various peptides (13, 14). Vancomycin is an effective drug for the treatment of infections with penicillin- and methicillin-resistant strains of Staphylococcus aureus (1, 9, 16). Drug resistance has not been demonstrated.

Therapeutic serum concentration during vancomycin treatment of Staphylococcus aureus infections varied from 10 to 60 µg/ml dependent upon the sensibility of the strain being treated. Minimum inhibiting concentration for Staphylococcus aureus is reported to be between 0.8 and 6.2 µg/ml (3). Ototoxicity has been seen with concentrations between 80 and 100 µg/ml (4). Development of renal insufficiency during vancomycin therapy has been described in severely infected patients and thus nephrotoxicity cannot be ruled out (1).

In individuals with normal renal function 90% of an administered dose of vancomycin is eliminated through the kidneys (8). In anuric patients adequate serum concentrations can be maintained by about 7% of normal dose (2, 11). The relationship between vancomycin clearance and glomerular filtration rate (GFR) has previously only been investigated in dogs in which the C_{Vanc}/C_{Cr} ratio was found to be 0.69 ± 0.05 (10).

In anuric patients in intermittent haemodialysis therapeutic concentrations of vancomycin could be maintained with 7% of the normal dose, which is in agreement with previous studies (2, 11).

In patients with varying degrees of renal insufficiency the rate of elimination of vancomycin was dependent on renal function and correlated well with the GFR expressed as inulin clearance and creatinine clearance. A negative limit correlation was demonstrated between C_{Vanc}/C_{In} ratios and serum albumin concentrations. In a patient with markedly reduced serum albumin concentration the C_{Vanc}/C_{In} ratio was close to 1.0 which means that C_{Vanc} was almost identical with GFR. Since vancomycin can form complexes with peptides in vitro these findings can be explained by partial binding of vancomycin to albumin in the serum and by glomerular filtration of the non-bound vancomycin. Using all data collected C_{Vanc} corresponded to 79%

of the GFR measured by C_{In}. This may have been due either to binding of vancomycin to serum proteins or reabsorption or backward diffusion of vancomycin in the kidneys Since studies of binding to serum proteins are not available the present study does not allow any clear-cut conclusion on this question

It can be concluded that C_{vanc} correlates well to the GFR and that the necessary maintenance dose of vancomycin can be calculated on the basis of this value thereby reducing the number of serum vancomycin determinations during treatment. Maintenance dose (μg/day) will thus be identical to C_{vanc} (ml/min)×1 440 (min/d)×therapeutic serum concentration of vancomycin (μg/ml) +7% of normal dose (μg/d)–C_{vanc}=C_{Cr}×0.53 or C_{In}×0 79

In renal insufficient patients vancomycin treatment can be carried out as follows A normal initial dose is given. Serum vancomycin measurements are necessary during initiation of treatment in order to be sure that a therapeutic level is reached When the therapeutic level has been obtained the maintenance dose can be calculated on the basis of the above mentioned formula. If a serum concentration of 20 μg/ml is desired the maintenance dose will approach C_{Cr}×0.53×1 440×20=(C_{Cr}×15) mg/d + 150 mg/d In anuric patients 7% of the normal dose will equal maintenance dose per unit time

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MONOCLONAL IMMUNOGLOBULINAEMIA ASSOCIATED WITH GLOMERULOPATHY

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Abstract Four patients with benign monoclonal immunoglobulinaemia and associated glomerulopathy are described. Immunohistochemical investigations of the immunoglobulin-containing cells in the bone marrow revealed an unexpectedly pronounced predominance of monoclonal over polyclonal cells as typically seen in macroglobulinaemia and multiple myeloma, but in contrast to the malignant plasma cell proliferations the percentage of immunoglobulin-containing cells only constituted 6-12% of the nucleated cells. The possible pathogenetic mechanisms relating monoclonal immunoglobulinaemia and glomerulopathy are unknown. The sera did not contain antibodies to glomerular basement membrane, cryoglobulins, antinuclear factors or antiglobulins. The immunohistochemical technique certainly offers clear advantages over conventional bone marrow cytology in the study of patients with monoclonal immunoglobulinaemia.

In 1966 Meltzer et al (10) described the association of essential cryoglobulinaemia and glomerulonephritis in three patients. Since then this combination has been reported by several investigators. In most cases the glomerulopathy has been post-streptococcal glomerulonephritis or rapidly progressive glomerulonephritis of unknown aetiology (1 11).

Monoclonal immunoglobulinaemia mostly without circulating cryoglobulins is found in multiple myeloma, Waldenström macroglobulinaemia and several other disorders, among which some are termed essential monoclonal immunoglobulinaemia because of lack of knowledge about an underlying cause. The latter disorder has only once been described in combination with glomerulonephritis by Kaplan and Kaplan (7).

In the present paper we report on four patients with essential monoclonal immunoglobulinaemia and glomerulopathy. Immunohistochemical analysis of the bone marrow was performed in each patient.

MATERIAL AND METHODS

Case histories

Patient 1 A 68-year-old male. The family history was not contributory. He had passed a renal stone 20 years ago. For the last 30 years he had suffered from muscle pain especially in cold weather.

In March 1970 he was admitted to the Medical Department, Rigshospitalet, because of a nephrotic syndrome. The proteinuria was 4-6 g/24 h, serum creatinine 1.5 mg/100 ml and endogenous creatinine clearance 79 ml/min. BP was 160/70 mmHg. Serum cholesterol and triglycerides were normal. A kidney biopsy showed membraneous glomerulonephritis. Serum contained an IgM-κ M-component in a concentration of 9 g/l, determined by paper electrophoresis, but no rheumatoid factors, antinuclear factors or cryoglobulins (12). No Bence Jones protein was found in the urine. The bone marrow contained 1% normal plasma cells and % lymphoid cells with an irregular nucleus and a narrow rim of strongly basophilic cytoplasm. X-rays of the skull and pelvis were normal.

Because of the nephrotic syndrome the patient was treated with prednisone 60 mg a day and azathioprine 100 mg a day for 4 weeks without any effect on proteinuria or the concentration of the M-component in serum. The patient was discharged on furosemide therapy. At the last control in March 1974 proteinuria was 3.4 g/24 h, serum creatinine .5 mg/100 ml and endogenous creatinine clearance 77 ml/min. BP was 200/100 mmHg on furosemide and methyldopa therapy. Serum concentration of the M-component was 5. g/l. The concentration of polyclonal Ig in serum determined by the method of Weeke (14) was normal (Table I). No hyperviscosity syndrome or other signs of Waldenström macroglobulinaemia were found.

Patient 2 A 53-year-old male. The patient s mother had had diabetes at the age of 60. At the ge of 4 a diagnosis of diabetes mellitus was suspected in the patient but no therapy was started In 1963 proteinuria was found. I 1966 the patient passed 3 renal stones

In March 1967 he was admitted to the Medical Depart ment Rigshospitalet, because of headache and fatigue He was obese BP 180/120 mmHg, serum creatinine 2.7 mg/100 ml and endogenous creatinine clearance 49 ml/min. A proteinuria of 5–10 g/24 h was found Urinary excretion of glucose 0–2 g/24 h. Fasting plasma glucose was normal. A percutaneous kidney biopsy showed lobular glomerulonephritis. *Serum contained an M-component* of type IgG-κ in a concentration of 13 4 g/l The same globulin (19% of total urine protein) plus Bence Jones protein was found in the urine. No rheumatoid factors antinuclear factors or cryoproteins were found in serum The bone marrow contained only 2% normal plasma cells. X-rays of the skull, pelvis and vertebral spine were normal.

The patient was treated with thiazides and methyldopa and was followed in the Outpatient Department. The kidney function deteriorated and in Nov 1973 chronic intermittent peritoneal dialysis was started. The concentration of the M-component in serum was at that time unaltered Serum concentration of polyclonal Ig was normal (Table I) and there were till no signs of multiple myeloma.

Patient 3 A 56-year-old male. No family history of renal disease diabetes mellitu or hypertension. At the age of 20 the patient was successfully treated with gold salts for rheumatoid arthritis At the age of 40 he wa treated for a period with glucocorticoids for the same disease. During the last 20 years the patient had consumed large amounts of analgesics because of joint pain. I 1966 proteinuria (2–3 g/24 h) was discovered Serum creatinine was normal

In Dec 1969 the patient was admitted to the Medical Rigshospitalet, in terminal uraemia Serum ne was 10 mg/100 ml and endogenous creatinine was about 8 ml/min Proteinuria was 4–5 g/24 h. was normal N subjective or objective signs from the joints were found A kidney biopsy was performed. A hi tological diagnosis was difficult to establish because of scarring of the kidney tissue but compatible with chroni poliferative glomerulonephritis. No signs of myelomatous kidney alterations or amyloidosis were found A biopsy of the rectal mucosa wa normal Serum contained an M-component of the type IgG-κ in concentration of g/l. No rheumatoid factors, antinuclear factors or circulating cryoglobulins were found in the serum. The urine contained the same M-component in an amount of 11% of the total protein excretion. No Bence Jones protein was found in the urine Examination of the bone marrow demonstrated 9% plasma cells, some of which were atypical with vacuolated nuclei and several nuclei per cell X-rays of the keleton were without signs of multiple myeloma.

The patient was treated with intermittent dialysis until March 1971 when he received cadaveric kidney At the time of transplantation the concentration of M-component *in serum was 6.2 g/l. Prednisone (initially 150 mg/day) and* azathioprine (100–200 mg/day) in combination with ex tracorporeal irradiation of the blood were given as immunosuppressive treatment.

The course was uneventful and at the last follow-up in April 1974 the patient was doing well with an endogenous creatinine clearance of 75 ml/min From the time of trans plantation until Oct. 1971 (i.e. 7 months) the urine con tained no protein, but from this date increased urine tein was seen thus t the last examination 7 1 g/24 h. Tl concentration of the M-component in serum was at tha time 9.6 g/l The same M-component was found in urine The bone marrow contained 8% plasma cells and as in the first bone marrow specimen some of these were atypical X-rays of the skeleton showed no signs of multiple myeloma. The concentration of polyclonal Ig was normal (Table I)

P tient 4 A 71 year-old female. Her daughter has chronic kidney disease For several years the patient had suffered from nocturnal dyspnoea and ECG had shown a WPW block.

In Aug 1971 the patient was admitted to the Medical Department Copenhagen County Hospital, with a nephrotic syndrome. Proteinuria was 8–12 g/24 h serum creatinine 2 0 mg/100 ml, and endogenous creatinine clearance 38 ml/min. Serum contained an IgM-κ M-component in a concentration of 9 g/l but no rheumatoid factors, antinuclear factors or cryoglobulins. The urine contained no Bence Jones protein. The bone marrow was normal with % plasma cells. X-rays of the skull ertebral spine and extremities were normal. No hyperviscosity syndrome or other signs of Waldenström s macroglobulinaemia were present. A kidney biopsy showed "minimal changes

The patient was treated with prednisone and had a complete remission of the nephrotic syndrome, and pred

Table I *Monoclonal and polyclonal immunoglobulins in serum (g/l) and histological findings in bone marrow*

Pat. no.	M-comp	IgG*	IgA	IgM	Histological findings in bone marrow
1	2.9	7 6	1 2	0.85	1% normal plasma cell and 5% lymphoid cells
2	13 4	11.2	1.0	0.67	?% normal plasma cells
3	12.0	17.6	0 61	0.88	9% plasma cells, some with vacuolated nuclei
4	10.0	8 6	0 6.	1 32	2% normal plasma cells

Determined electrophoretically Determined immunochemically

nisone was withdrawn in Nov 1971 I May 197? the patient was admitted to a surgical department and cholecystectomy was performed. During this admission Albustix® was positi but no further xaminations were done. The patient was discharged and readmitted in July 1972 with a relapse of the nephrotic syndrome.

Prednisone treatment wa resumed this time without success. Proteinuria persisted in an amount of 2–3 g/24 h Therefore in May 1973 the treatment was supplemented with cyclophosphamide (150 mg a day). In June the same year the urine became free of protein and prednisone was withdrawn in Dec. as was cyclophosphamide in March 1974 At this last follow-up serum creatinine was 1 1 mg/100 ml endogenous creatinine clearance 41 ml/min. BP was 150/70 mmHg. The urine contained no protein and the urinary sediment was normal. Polyclonal Ig were normal (Table I)

Special investigations

Immunohistochemical studie of bone marrow Bone marrow specimens were studied by direct immunofluorescence technique according to the method of Hijmans et al (5). Unlabelled and fluoresceinisothiocyanate (FITC)-labelled rabbit IgG specific for human α, μ, γ and λ chains were purchased from Dakopatts A/S, Copenhagen. The unlabelled rabbit antibodies were labelled with tetramethylrhodamineisothiocyanate (TMRITC) as described by Cebra and Goldstein (2). Before se all conjugates were tested for specificity by crossed immunoelectrophoresis (8) and by performance tests using monoclonal bone marrow cells from patients with multiple myeloma and macroglobulinaemia (15).

I all patients double staining xperiments were done to determine in the same preparation whether the cell containing one particular type of heavy chain also contained only one type of light chain, the maximal criterion for calling the plasmocytic proliferation monoclonal.

All cells containing a particular heavy chain type were counted in each cytocentrif ge slide preparation and subsequently the relative percentages were calculated The κ/λ ratio was always counted in one slide preparation stained with FITC-anti-κ and TMRITC-anti-λ.

Antiglomerular basement membrane antibodies Cryostate sections of normal human kidney tissue (4–6 μ thick) served as substrate for detection of antiglomerular basement membrane antibodies by indirect immunofluorescence technique Sera were screened at dilutions 1 1 1 4 and 1 16 ng a 1 50 diluted FITC labelled rabbit antiserum specific for human α μ, γ and λ chains (Dakopatts A/S Copenhagen).

RESULTS

In patients 1 and 4 with an IgM κ M-component in serum 83% of the monoclonal bone marrow cells contained μ chains and 96 and 72% respectively contained κ-chains In patients and 3 with an IgG-κ M-component in serum the corresponding figures were 98 97 and 98% respectively

Ig-containing cells as a percentage of nucleated cells were determined in 3 patients and varied from 6.3 to 12.2. In patients 1 and 4 the Ig-containing cells were predominantly lymphoid, and in patients 2 and 3 mainly plasmocytoid (Table II) No antiglomerular basement membrane antibodies were found in any of the patients

DISCUSSION

In addition to patients with multiple myeloma a d Waldenström s macroglobulinaemia, monoclonal immunoglobulins are encountered among patients with cancer amyloidosis and leukaemias These diseases were excluded on clinical or histological grounds in the present patients

As monoclonal Ig in plasma can be found in apparently healthy persons especially in old age (6 13) there could have been a chance association between benign monoclonal immunoglobulinaemia and kidney disease in our patients However it is remarkable that all the patients suffered from a glomerulopathy and not from different renal disorders. Furthermore such an association would be rare considering the low incidence of both diseases.

Therefore it is tempting to postulate some kind of

Table II *Immunohistochemically determined relative perc ntages of bone marrow cells containing γ α and μ heavy chains and κ and λ light chains Morphological feature*

Pat no.	Cells containing heavy and light chains (%) γ	α	μ	κ	λ	Ig-containing cells 100 nucleated cells	Characteristics of monoclonal cells
1	15	2	83	96	4	12 2	Mainly small lymphoid cells with narrow m of cytoplasm
2	98	1	1	98	2		Mainly plasmocytoid ells
3	97	2	1	98	2	9 0	Mainly plasmocytoid cells
4	12	5	83	72	28	6 3	Both plasmocytoid and lymphoid cells

causal relationship between the monoclonal immunoglobulinaemia and the glomerulopathy At our present state of knowledge the two pathogenetic mechanisms for the induction of glomerulonephritis should be considered one invol ing type II reaction between antiglomerular basement membrane antibodies glomerular basement membrane and complement leading to damage of the membrane the other involving type III reaction due to immune complex deposition in the glomeruli The latter mechanism is thought to be the more common We cannot say which of the two mechanisms is the most likely in our patients, since no immunohistochemical studies were performed on the renal biopsies The only earlier report of monoclonal immunoglobulinaemia accompanied by glomerulopathy (7) did not include immunohistochemical studies either The lack of antiglomerular basement membrane antibody activity in the sera does not exclude type II reactions as such antibodies may be completely trapped in the glomeruli (9)

Serum electrophoresis did not indicate aggregate formation of the M-components but this does not exclude that the M-component might participate in immune complexes

Monoclonal immunoglobulinaemia in combination with rheumatoid arthritis has been described by several authors (3 16) The M-component often showed antiglobulin activity In our rheumatoid arthritis patient (no 3) such activity of the M-component was not detected However the possibility remains that the M-component might have activity against circulating autologous antigens

Finally the M-component might itself act as foreign antigen triggering antibody production and immune complex formation As none of the patients had circulating cryoglobulins the pathogenetic mechanism may be different from that hypothesized by Skrifvars et al (11)

In the immunohistochemical investigations of the bone marrow the percentage of nucleated cells containing Ig was increased compared to normal persons, but not to an extent found in malignant proliferation of plasma cells or lymphoid cells as seen in multiple myeloma or macroglobulinaemia However the monoclonal population constituted a very high percentage of the Ig-containing cells quite similar to that found as a rule in malignant cell proliferation (4) The last finding compared to the relatively low quantities of M-component in the corresponding sera suggest that either 1) the monoclonal cells secrete less Ig than polyclonal cells 2) the M-component is hypercatabolized or excreted very fast, or 3) most of the polyclonal Ig is produced in lymphoid organs outside the bone marrow At present none of these possibilities can be excluded although the last one seems the most likely

The results of conventional histological and immunohistochemical investigation of the bone marrow in this study shows that only the latter investigation gives a true picture of the activity of the bone marrow in producing M-components in monoclonal immunoglobulinaemias.

The experience gained from this study seems to indicate that both the percentage of Ig-containing cells among the nucleated cells and the relative percentage of the Ig-containing cells constituted by M-component-containing cells are relevant in attempting to differentiate between benign and malignant plasma cell proliferations Obviously longitudinal studies of these parameters are even more important for this distinction

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THE EFFECT OF NOREPINEPHRINE AND THEOPHYLLINE ON BLOOD GLUCOSE PLASMA FFA PLASMA GLYCEROL AND PLASMA INSULIN IN NORMAL SUBJECTS

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Abstract The plasma concentrations of free fatty acids (FFA), glycerol and insulin as well as the blood glucose concentration have been followed in two groups of subjects after infusions of theophyllamine. Each individual was examined twice. The 5 subjects i group I were given an infusion of norepinephrine before the theophyllamine at one of the examinations and saline at the other The 6 subjects in group II were given an infusion of norepinephrine at both examinations, followed by theophyllamine on one occasion and by saline on the other Thus, the subjects in both groups served as their own controls. It was found that theophyllamine caused lipid mobilization, as measured by the plasma FFA and plasma glycerol concentrations, both when gi en as the only active drug and when given after norepinephrine. The blood glucose concentration rose slightly after norepinephrine and the plasma insulin level increased concomitantly When theophylline was given as the only active drug, there was no increase in the blood glucose but the plasma insulin concentration rose slightly

Theophylline has been shown to exert a stimulating effect on the lipolytic process in adipose tissue in animals (2) and on human adipose tissue in vitro (3)

According to the current theory the lipolytic effect is mediated through inhibition of the phosphodiesterase activity which enzyme converts cyclic AMP (cAMP) to inactive AMP Theophylline thus, increases the cAMP concentration by diminishing its breakdown and as the cAMP concentration controls the rate of lipolysis conse quently stimulates lipolysis.

The catecholamines, on the other hand exert an effect on lipolysis by stimulation of the adrenergic receptors in adipose tissu causing an activation of the enzyme adenylcyclase, which converts ATP to cAMP As a result, the concentration of cAMP is increased and lipolysis stimulated Thus theophylline and catecholamines both increase the cAMP concentration, but their effects are mediated via separate enzymatic mechanisms and it is theoretically possible to expect an additional effect when both drugs are given.

The present study was started as trial to elucidate 1) if theophylline in vivo has an effect on the lipolysis and the lipid mobilization, as measured by the plasma free fatty acid (FFA) and the plasma glycerol concentrations and 2) if theophylline in vivo further increases the lipid mobilization nduced by norepinephrine. Preliminary results of this study have been published earlier (5)

MATERIAL AND METHODS

Eleven apparently healthy male volunteers, aged 23–44 years, were studied. The subjects were divided into group I ($n=5$ mean age 27 years) and group II ($n=6$ mean age 30 years) and each individual was examined on two occasions on different days.

The examination started in the morning with the subjects in the fasting state. One catheter was inserted into the brachial artery and one into one of the cubital veins After about one hour' rest infusions of the drugs were given via the venous catheter and blood samples were drawn through the arterial catheter The drugs were infused during tw consecutive periods of 10 min each whereafter the subjects rested for 60 min.

The subjects in group I were on one occasion given 75 μg norepinephrine during the first 10 min and 10 ml theophyllamine ACO (theophylline 23 mg/ml) during the following 10 min. On the other occasion the same individuals were given physiological saline during the first period and the theophyllamine during the second period. Thus, the individuals served as their own controls.

I group II the subjects were given 75 μg norepinephrine

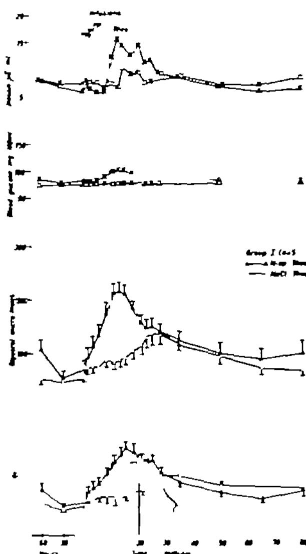


Fig. 1. Plasma FFA, plasma glycerol, blood glucose and plasma insulin concentrations during the experiment in group I. Mean ± S.E.M.

during the first infusion period on both occasions. It was followed by 10 ml theophyllamine ACO the second infusion on one occasion and by physiological saline on the other. In this group too the subjects served as their own control.

During the whole experimental period ECG was recorded and direct measurements of the intraarterial BP were made at intervals. Blood samples were drawn at 60 and 30 min before, at the start of and at 1, 3, 5, 8, 11, 13, and 18 min during the infusions. After the infusions blood samples were drawn at 1, 23, 25, 28, 35, 50, 65 and 80 min. The blood was collected in test tubes containing heparin as anticoagulant.

The blood samples were kept in ice water and plasma was separated within 90 min. The whole experiment was performed in the Department of Clinical Physiology, University Hospital of Lund, Sweden.

Plasma FFA was titrated according to Trout et al. (13). Plasma glycerol was determined according to Laurell and Tibbling (9) and blood glucose according to Mark (11). Plasma insulin was estimated with the method of Hedin (7).

RESULTS

The plasma FFA, plasma glycerol, blood glucose and plasma insulin concentrations in group I during the experiment are illustrated in Fig. 1. It is apparent that the norepinephrine and theophylline infusions increased the concentrations of plasma FFA, plasma glycerol and blood glucose. The plasma insulin level rose slightly, parallel to the increase in blood glucose.

When the individuals in group I were given saline as the first infusion, the following theophylline infusion caused a rise in the plasma FFA and the plasma glycerol, blood glucose and plasma insulin concentrations in group II. When norepinephrine was given ... showed no variations. The plasma insulin level rose slightly but significantly.

Fig. shows the mean plasma FFA, plasm

Table I. *Hemodynamic data*

Combinations of infusions	Before infusion BP S	D	M	HR	During infusion I BP S	D	M	HR	During infusion II BP S	D	M	HR	After infusion BP S	D	M	HR
Group I (n=5)																
N-ep+Theo	116	71	91	60	143	83	108	48	123	68	90	64	122	73	93	6_
NaCl+Theo	114	67	85	52	116	66	85	53	122	71	90	56	1?6	73	96	61
Group II (n=6)																
N-ep+Theo	109	68	82	56	135	69	103	49	114	66	84	60	115	64	87	58
N-ep+NaCl	111	65	83	53	131	78	100	46	115	62	83	55	114	67	83	56

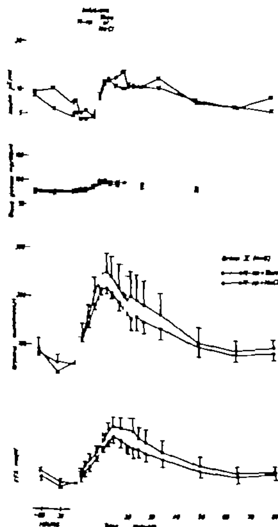


Fig 2 Plasma FFA, plasma glycerol blood glucose and plasma insulin concentration during the experiment in group II. Mean ±S.E.M.

glycerol blood glucose and plasma insulin concentrations in group II When norepinephrine was given as the only active drug, followed by saline there were rises in plasma FFA plasma glycerol and blood glucose of the same magnitude as in group I with the same infusions. When however the norepinephrine infusion was followed by theophylline the rises in plasma FFA and plasma glycerol were somewhat more pronounced, lasted longer and showed tendency towards two maxima.

The blood glucose curve was the same on both occasions in group II Both curves showed a slight rise and a concomitant slight ncrease n the plasma insulin level.

The hemodynamic data are summarized in Table I In group I the mean intraarterial BP rose and the heart rate decreased when norepinephrine was given as the first infusion. When saline was given, no variations were noticed. The subjects i group II showed on both occasions a rise in the mean arterial BP and a bradycardia following the norepinephrine infusion

The theophylline infusion caused no variations in the hemodynamic parameters in either group

DISCUSSION

These studies indicate that theophyllamine is an active stimulator of lipolysis and lipid mobilization in vivo when measured as the plasma concentrations of FFA and glycerol The response to theophyllamine was delayed as compared to the norepinephrine response This supports the view of dissimilar mechanisms for the effect of norepinephrine and theophyllamine on the lipolysis. If one accepts the theory that their effects are related to an increased production rate of adenylcyclase by the former and a decreased degradation rate by the latter one would expect the combined use of both agents to result in exaggerated responses regarding FFA and glycerol This wa the case and furthermore, these combined responses had a 2-phase character as if the two stimulants worked in an unrelated manner

Theophyllamine given as the only drug induced a small rise in insulin in spite of the absence of changes in blood glucose. It is known that agents which raise the concentration of cAMP in the B cell enhance the glucose- timulated release of insulin (10), though this effect is not seen in the absence of glucose. But the marked increase in FFA may have exerted an influence as well It has been reported that experimental elevation of FFA in dogs stimulates the re lease of insulin (6). In man FFA alone does not stimulate to increased insulin secretion (12) but may enhance the response to other stimuli (1) Combined enhancing effects of cAMP and FFA may therefore cause a sensitivation of the B cells for glucose to the extent that the basal concentration of glucose is high enough to induce an increased release of insulin. The even higher insulin response noted in the nor epinephrine experiments when the glucose level rose slightly is then probably just an exaggeration of the same effect

The lipolytic response as well as the dynamic data when norepinephrine

as the only active drug, were in agreement with earlier findings (4). Theophylline showed no hemodynamic effects.

Theophylline is commonly used in the treatment of asthma bronchiale and cardiac insufficiency. Judging from the present results it is able to cause an elevation of the plasma FFA concentration. Kurien et al. (8) have proposed that raised plasma FFA levels may induce arrhythmias in patients with myocardial infarction. If their theory is correct, theophylline should perhaps be avoided in the treatment of cardiac insufficiency in patients with myocardial injury.

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PORTAL AND CUBITAL SERUM INSULIN DURING ORAL, PORTAL AND CUBITAL GLUCOSE TOLERANCE TESTS

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Abstract Oral intracubital and intraportal glucose tolerance tests have been performed on 7 non-obese non-diabetics, and glucose and insulin concentrations have been followed in the peripheral and portal blood A significant rise in portal glucose and insulin was found 1–2 min after oral glucose intake There was no lag between the rise in insulin and glucose concentrations The portal glucose concentration after oral glucose intake was significantly higher than after cubital glucose infusion for 45 min, although the peripheral glucose concentrations were identical. In the cubital vein the insulin concentration after oral glucose intake was significantly higher than after i v glucose infusion, but in the portal blood there was no difference. After portal glucose infusion the cubital insulin concentration did not differ significantly from the concentration after i v glucose infusion. Thus, it seems unlikely that a high portal glucose concentration is responsible for the higher peripheral insulin concentration after oral glucose intake A high portal glucose concentration does not seem to influence the hepatic uptake or release of glucose.

Oral intake of *d*-glucose is known to be followed by a stronger insulin response than a corresponding i.v. glucose infusion (13) but it is not clear which factors are responsible for the difference. Various intestinal hormones—gastric inhibitory peptide (5) secretin (4) gastrin (18) entero-glucagon (15 22) and insulin-releasing polypeptide (21)—have been suggested, but nervous stimuli (8 16 20) and hepatogenic factors (7) have been mentioned as well

The present study was designed to investigate whether hepatogenic factors play a role in the potentiated insulin response in man following oral intake of glucose In other words whether a high portal glucose concentration is able to increase the secretion or decrease the elimination of insulin

It has been demonstrated by Goetz et al (7) that intraportal infusion of glucose to the dog increases the insulin secretion without any change in the glucose concentration in the systemic arterial blood indicating an extrapancreatic glucose-sensitive regulatory mechanism Kaplan and Madison (10) found hepatic extraction of insulin [131]I to fall with rising glucose concentration in the liver indicating a reduced hepatic elimination of insulin at high portal glucose concentrations

We therefore studied glucose and insulin concentrations in the portal and cubital vein of non-diabetics during oral intracubital and intraportal glucose tolerance tests (GTT)

MATERIAL AND METHODS

The material comprises nine patients, tested on 3 different days, 5–10 days after laparotomy involving cholecystectomy in seven and exploratory operations because of pain in two Seven of the patients (6 females and 1 male) were non-diabetics and non-obese aged 31–66 years (mean 53) Two patients were ultimately excluded as their blood sugar levels exceeded 150 mg/100 ml 120 min after oral administration of glucose During the operation a "baby feeding tube" was inserted into the portal vein through the obliterated umbilical vein. The catheter was kept open by constant infusion of heparinized saline At the time of the investigations all the patients were fully mobilized and had normal intestinal hepatic and renal function

On the first test day the patients received 90 g *d*-glucose by mouth after 12 hours fasting Blood samples were drawn simultaneously from the cubital vein, portal vein, and ear capillaries Capillary plasma glucose, venous serum glucose from the cubital and portal veins as well as venous serum insulin from the cubital and portal veins were determined at the times shown in Figs 1–4

On the second test day glucose infusion was given through a cubital vein, and the same parameters were investigated

On the third test day glucose infusion was given

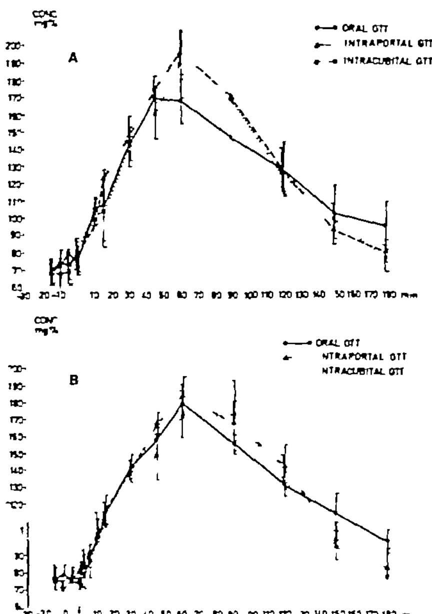


Fig 1 Plasma glucose in capillary (*A*) and cubital blood (*B*) (mean ±S E M)

through the portal vein catheter Simultaneous blood sampling for determining portal glucose and portal insulin was not possible, but otherwise glucose and insulin were determined as on the first test day The glucose infusion rate was adjusted so that the glucose concentrations in the peripheral blood were comparable to those obtained with the oral glucose load

On all blood samples from the portal vein, haematocrit was determined to rule out dilution from the saline infusion. Oxygen saturation in the portal blood was determined periodically as a relative measure of the flow variations of the portal blood. Determination of glucose was performed by the glucose oxidase method on Beckman's auto-analyser Immunoreactive insulin was determined by the solid phase immunoassay technique of Wide (24). The intra-assay coefficient of variation was 8.5% inter-assay coefficient of variation 12.4% The difference between insulin and glucose concentration during the various tolerance tests was assessed by Student *t*-test for paired data

RESULTS

Fig 1 gives the plasma glucose concentrations in ear capillary blood and in cubital blood during the oral intracubital and intraportal GTT It is evident that during the i v loads it was possible to attain glucose concentrations of the same course as those during the oral glucose loads

From Fig it is apparent that although the glucose concentration peripherally (in ear blood and in the cubital vein) did not differ significantly during

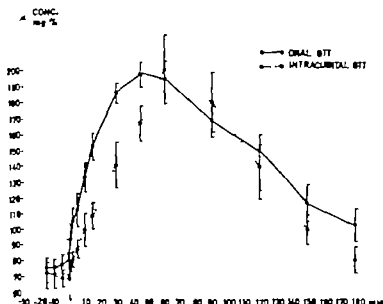


Fig. 2. Plasma glucose in portal blood following oral and intracubital glucose load (mean ± S.E.M.).

the oral and the i.v. glucose loads (Fig. 1) there was a considerable difference from the glucose concentration in the portal vein. In the portal blood the glucose concentration rose abruptly immediately after the oral intake of glucose, and as early as 3 min after the intake it had increased by 34 mg/100 ml. The portal glucose concentration during this test proved to be significantly higher for up to 45 min than during the cubital test (Fig. 2).

The mean insulin concentration in serum from the cubital and portal veins during the tests is shown in Figs. 3 and 4. From Fig. 3 it is apparent that the insulin concentration in the cubital vein was significantly higher after oral than after intracubital administration of glucose.

Cubital vein insulin concentration during intraportal glucose infusion showed marked variations. In patients showing almost complete agreement between the glucose concentration peripherally during the course of all three tolerance tests

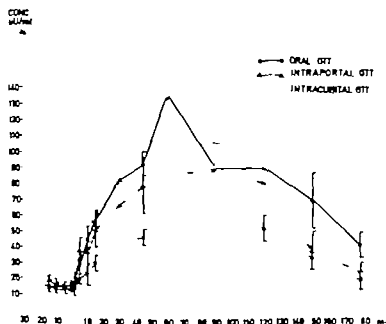


Fig. 3. Plasma insulin in cubital blood following oral, intraportal, and intracubital glucose loads (mean ± S.E.M.). ★ = significantly different values following oral and intracubital administration of glucose.

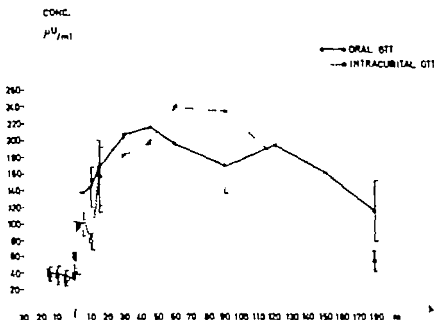


Fig. 4. Plasma insulin in portal blood following oral and intracubital glucose load (mean ± S.E.M.).

the cubital vein insulin concentration in the portal tolerance test was identical with that during the cubital tolerance test and lower than that during the oral test (Table I) In others the cubital vein insulin concentration was on a few occasions higher during the portal than during the oral load These high values were invariably preceded by higher peripheral glucose concentrations On an verage the insulin concentration in the cubital n was higher following portal than following cu- tal infusion of glucose However the difference was not ignificant The insulin concentration in the cubital ein following portal infusion of glucose was also not significantly lower than that following oral administration of glucose

Fig. 4 sets out the insulin concentrations in the portal blood during i.v and oral glucose loads Unlike the findings in the cubital vein there was no difference in the insulin concentration in portal blood apart from the 10 min after the commencement of the infusion which marks the well known biphasic insulin response during infusion of glucose

DISCUSSION

The object of the procedure was to attain a uniform glucose concentration around the β-cells after the different routes of administration in order thereby to secure a uniform glucose-induced insulin re sponse From Fig 1 (*A*) it may be seen that the

Table I *Plasma glucose and insulin in two patients with nearly identical glucose concentrations during the glucose infusions*

	Glucose concentration in capillary blood (mg/100 ml)						I sulin in cubital plasma (µU/ml)					
Time (min)	0	15	30	45	60	120	0	15	30	45	60	170
Patient 3												
Oral glucose	79	114	146	186	178	110	10	43	59	135	90	60
Intravenous glucose	72	96	155	185	10	148	79	16	3	29	31	40
Intraportal glucose	82	112	158	199	215	147	10	13	28	33	34	4.
Patient 5												
Oral glucose	79	115	139	175	198	136	16	65	99	103	795	94
Intravenous glucose	85	123	152	164	226	150	16	41	48	57	70	79
Intraportal glucose	87	122	160	190	230	178	16	30	41	47	58	105

mean plasma glucose concentration in the capillaries of the earlobe did not differ after the different forms of administration. Provided the blood flow in the capillaries of the islet tissue does not alter its direction with the different modes of administration the capillary plasma glucose concentration around the β-cells must also be considered to have been comparable after different routes of administration. Accordingly a difference in the cubital serum insulin concentration must be due to non-glucose-induced changes in the secretion or/and elimination of insulin Fig. 4 shows that the insulin concentration in the portal vein during oral and cubital OTT did not differ although the cubital insulin concentration following oral administration was significantly higher than after cubital glucose infusion. This might indicate the same insulin *secretion* during both tolerance tests but a reduced *elimination* of insulin in the liver during the oral glucose load.

It is possible, however that the blood flow in the portal ein during oral glucose load is greater than during an i.v glucose load. At least it has been demonstrated by Arnould et al (1) that the splanchnic flow in dogs increases during oral glucose load Kaden et al (9) however found an unchanged portal blood flow in dogs following intraduodenal administration of glucose. During an i. glucose infusion the blood flow in the pancreas does not alter (12) We were unable to measure the blood flow in the portal ein in our experimental design but measurement of the oxygen saturation of portal blood showed no systematic variations (86–91% after all routes of administration). Accordingly we are unable to answer the question whether insulin secretion during oral glucose load is increased or unchanged as compared with insulin secretion during the cubital glucose load but we considered it most likely that the portal blood flow is increased during oral glucose intake. However an unchanged portal insulin concentration at an increased flow in the portal vein during oral glucose load must signify an increased secretion of insulin.

Whether elimination of insulin i reduced during oral glucose intake is a question for future studies Genuth (6) was unable to demonstrate changes in insulin elimination during i glucose tolerance tests and Kaden et al (9) demonstrated an unchanged or increased insulin elimination i dogs during duodenal GTT There are no accessible reports elucidating the elimination of insulin in man during oral glucose loads. Kaplan and Madison s studies (10) have been criticized (9)

Thus, while it is likely that insulin secretion during oral glucose loads is greater than during cubital glucose loads it has not been elucidated whether high portal glucose concentration plays any role in this connection

The present study has shown that the portal glucose concentration is significantly higher during oral than during cubital administration of glucose Therefore it cannot be rejected that a high portal glucose concentration is responsible for the difference in cubital insulin concentration observed after oral and i.v administrations respectively To prove or disprove this the above mentioned portal glucose infusions were performed, in which the amount of glucose and rate of infusion were identical with those of the cubital glucose infusion It was not technically possible to measure the portal glucose concentration during the portal infusion of glucose but it must be assumed that this was considerably higher than during the cubital nfusion. However it cannot be decided whether it was also higher than during the oral glucose load but this must be considered likely

A high portal glucose concentration such as that obtained during portal infusion of glucose did not, however result in a significantly higher cubital insulin concentration (Fig. 3) This agrees with studies dogs by McIntyre et al. (14) who found identical insulin concentrations peripherally after portal and after peripheral glucose nfusion. White and Dupré (13) too ha e demonstrated with a few case reports that portal glucose infusion does not lead to a higher cubital insulin concentration than does cubital infusion of glucose

The cubital insulin concentration during portal infusion of glucose was not, however significantly lower than during oral glucose load in our studies True, the individual results varied widely as it was not possible to attain identical capillary plasma glucose concentrations in all the subjects during the different routes of administration. But if the successful tests are considered separately (Table I) it is striking that the cubital insulin concentration during portal infusion coincided with this concentration during cubital infusion but not with the concentration during oral administration.

It is unlikely therefore that the potentiated insulin response following oral administration cose i due to mechanisms elicited by

glucose concentration Gastrointestinal hormones or neurogenic mechanisms elicited from the gastrointestinal tract must still be considered the most important factors in potentiating insulin secretion following oral administration of glucose This appears even more likely as potentiation of insulin secretion follows not only upon oral administration of *gl cose* but also upon oral administration of amino acid (17) and fat (2) According to the findings made so far the gastrointestinal hormone which seems the most likely candidate for potentiating insulin secretion during oral GTT is the gastric inhibitory peptide (GIP) as injection of GIP causes an increase in insulin concentration and as the serum GIP concentration rises during oral but not during i v GTT (5) However it cannot be ruled out that other gastrointestinal hormones are operative too

Neurogenic mechanisms elicited from the intestinal canal are not likely to play a major role in man considering that Lund et al (11) found the same potentiating effect of orally administered glucose upon the insulin response in patients before and after total vagotomy

Lastly an unintended result of the present study must be pointed out because it concerns a problem which has been widely discussed. This is the question whether the portal glucose concentration alone is able to regulate the glucose uptake and/or glucose release in and from the liver independently of variations in the insulin and glucagon concentrations The finding that the capillary plasma glucose concentration is the same after portal and after cubital infusion of glucose indicates that the greater amount of glucose presented to the liver during the portal infusion of glucose does not alter the regulatory mechanisms of the liver in man. Similar findings have been made by McIntyre et al (14) in the dog. Thus whereas the liver does not seem to exhibit any autoregulation in the case of major acute changes in plasma glucose, it cannot be ruled out that the liver exerts an autoregulatory function on minor variations in blood glucose (3 19)

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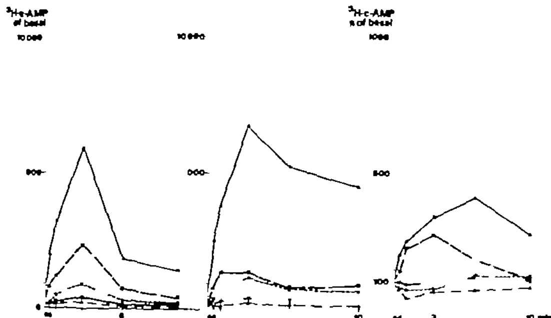


Fig. 2. Effects of noradrenaline (2×10^{-6} M), phentolamine (5 μg/ml) and theophylline (10^{-3} M) on [^{3}H] cyclic AMP accumulation during 0.5–10 min of incubation. Results from 3 individual experiments are expressed as % of [^{3}H] cyclic AMP in samples incubated under basal conditions. Δ = noradrenaline □ = noradrenaline and phentolamine ▲ = noradrenaline phentolamine and theophylline ■ noradrenaline and theophylline ○ = theophylline. Each point represent the mean of duplicates. Not the two different scales on the ordinat.

Short-term (30 sec) incubations

Effects of noradrenaline. The effects of noradrenaline on [^{3}H] cyclic AMP accumulation during 30 sec of incubation were tested. A very small stimulatory effect was observed when concentrations of 0.2×10^{-6} M and 2×10^{-6} M were used (Fig. 3) while 0.002×10^{-6} M and 0.02×10^{-6} M were without effect (data not shown).

Effects of phentolamine. The addition of phentolamine (0.005–5 μg/ml) potentiated the accumulation of [^{3}H] cyclic AMP induced by noradrenaline, the stimulation being more marked with increasing concentrations of the catecholamine (Figs. 3 and 5). Phentolamine per se tended to slightly depress basal [^{3}H] cyclic AMP levels (Fig. 3).

Effects of propranolol. The stimulation of [^{3}H] cyclic AMP accumulation induced by $\times10^{-6}$ M noradrenaline alone or in the presence of 5 μg/ml phentolamine was inhibited by 0.10–10 μg/ml propranolol, a total inhibition being obtained at concentrations of 1 μg/ml and above (Fig. 4).

Effects of theophylline. The addition of 10^{-4}–10^{-3} M theophylline significantly stimulated the accumulation of [^{3}H] cyclic AMP and magnified the stimulatory action of other agents (Figs. 3 and 5). The effect of 10^{-} M theophylline alone was not significantly modified by the addition of phentolamine, 5 μg/ml (−9.0±12.0% mean ±S.E.M. of difference in 11 experiments).

DISCUSSION

The present experiments have demonstrated rapid initial stimulation of [^{3}H] cyclic AMP, peak levels being reached within 3–6 min, followed by declining levels of the nucleotide (Fig. 2). This pattern of response could not be markedly altered by theophylline, a known phosphodiesterase inhibitor, nor by the α-adrenergic antagonist, phentolamine. These observations are in accordance with those of Manganiello et al. (16) and Ho and Sutherland (12) on fat cells from epididymal adipose tissue of rats. Ho and Sutherland presented data indicating that the decline or leveling off from peak cyclic AMP levels in adipose tissue was due to the generation and excretion of a hormone antagonist induced by elevated intracellular levels of cyclic AMP in the adipocyte. It is not known at present whether the above mentioned antagonistic substance decreases the

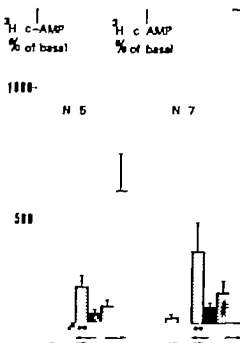


Fig 3 Effect of noradrenaline theophylline (1×10^{-4} M) and phentolamine (5 µg/ml) in 30 sec incubations. Noradrenaline 0.2×10^{-6} M (left) or 2.0×10^{-6} M (right) was added to incubations alone or together with theophylline or phentolamine. Columns from left to right in each part of the figure indicate incubations with noradrenaline phentolamine noradrenaline plus phentolamine, theophylline noradrenaline plus theophylline and noradrenaline plus theophylline plus phentolamine, respectively N = number of experiments (each performed with duplicate or triplicate incubations) Student s t-test for paired differences was employed ($p<0.05$ $p<0.01$) P-values between arrows = comparisons between columns indicated ly under columns = significance of difference from values. Vertical bars = S E.M.

synthesis or enhances the breakdown of cyclic AMP or both Since one aim of the present study was to evaluate how synthesis and breakdown of cyclic AMP are affected by α-adrenergic receptors the experiments were designed so as to minimize the influence of the above inhibitory mechanism Since accumulation of [³H] cyclic AMP was shown to be approximately linear during the first 30 sec of incubation (Fig 1) it was assumed that the adenyl cyclase activity is constant during this period Therefore 30 sec incubations were used in the following experiments

In order to evaluate whether the decrease in [³H] cyclic AMP induced by α-adrenergic stimulation was caused mainly by the stimulation of cyclic AMP breakdown, the effect of theophylline a known phosphodiesterase inhibitor was studied It was assumed that if α-adrenergic receptor activity stimulated phosphodiesterase activity theophylline would probably inhibit this effect The validity of such an assumption is strengthened by the observation that theophylline can inhibit the stimulatory effects of other substances e.g. cyclic GMP on phosphodiesterase enzymes (2) Our results show firstly that the effect of theophylline on [³H] cyclic AMP response to noradrenaline did not mimic the response to phentolamine (5 µg/ml) in the presence of noradrenaline (Fig. 3) Secondly when different concentrations of phentolamine were used together

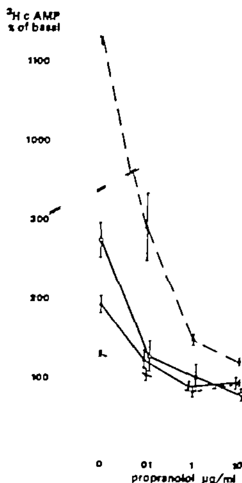


Fig 4 Effects of propranolol (0 1 10 µg/ml) on the [³H] cyclic AMP response induced by noradrenaline ($2\cdot10^{-6}$ M) in the presence or absence of phentolamine (5 µg/ml) and/or theophylline ($1\cdot10^{-4}$ M) Incubation time 30 sec Results are expressed as % of basal values. △ = noradrenaline plus phentolamine plus theophylline ○ = noradrenaline plus phentolamine ▲ = noradrenaline plus theophylline, ● = noradrenaline Each point represents the mean ±S E M from 2 experiments

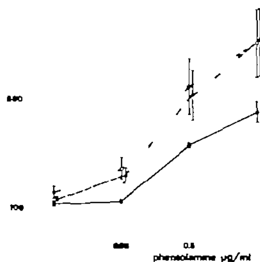


Fig. 5. Effects of phentolamine (0.05–5 μg/ml) on the [³H] cyclic AMP response to noradrenaline (0.2×10⁻⁶ M) in the presence or absence of theophylline (1×10⁻⁴–10⁻⁶ M). Incubation time 30 sec. Results are given in % of basal values. — noradrenaline plus phentolamine; -O- -- noradrenaline plus phentolamine plus theophylline (1 10⁻⁶ M); ▲ --- noradrenaline plus phentolamine plus theophylline (1×10⁻ M). Each point represents the mean ±S.E.M. of three experiments.

with noradrenaline the concomitant addition of theophylline seemed to augment [³H] cyclic AMP to the same degree regardless of the presence of varying α-adrenergic blocakde (Fig. 5). These results indicate that theophylline does not act as an α-adrenergic antagonist. Instead theophylline seems to have a multiplicative effect on the rat of [³H] cyclic AMP accumulation.

In conclusion our findings would seem to favour the hypothesis of α-adrenergic stimulation being mediated by inhibition of adenyl cyclase. It is realized however that an effect of α-adrenergic agents on cyclic AMP breakdown cannot wholly be excluded since theophylline does not block completely the phosphodiesterase enzymes.

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REMOVAL OF EXOGENOUS TRIGLYCERIDES IN HUMAN FOREARM MUSCLE AND SUBCUTANEOUS TISSUE

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Abstract The removal of exogenous triglyceride (TG) in forearm muscle and subcutaneous tissue of 7 healthy male volunteers has been studied by nephelometric determinations of arterial–deep venous (a–dv) and arterial–superficial venous (a–sv) differences in concentration of fat particles. Exogenous TG was administered as constant i infusion of Intralipid® over period of 30 min at rest and another 15 min during forearm work. At rest a significant positive a–dv difference in fat particle concentration of 121±21 μmol TG/l (mean±S.E.M.) was found, which corresponds to frac tional extraction of 10.3±2.0% Also the a–sv difference was significant 81±14 μmol TG/l 5.7±0.8% of the arterial concentration. During exercise no further significant removal was found. Thus both skeletal muscle and subcutaneous tissue seem to be able to remove exogenous TG in substantial amounts under resting conditions. In the exercising muscle, however the direct removal of exogenous TG does not seem to be of significant importance.

Exogenous triglycerides (TG) both in the form of chylomicrons and as an artificial fat emulsion given intravenously are rapidly cleared from the blood. Chylomicron removal in the whole body has been studied in several species such as rat (9 10) and dog (4 20) and also in man (13) In general it follows zero order kinetics above cer tain plasma concentration and first order kinetics below that level (4) In the lower concentration range 50% of isotopically labelled chylomicrons are removed from the blood after 5–12 min (9 22). At this point of time after injection most of the label is found in the liver adipose tissue and skeletal muscle but also in the heart a significant amount is found (2, 10) Whereas chylomicrons are believed to be removed intact by the liver hydrolysis by lipoprotein lipase seems to be a prerequisite for the extraction of chylomicron fatty acids by adipose tissue, heart and skeletal muscle (21 22).

Chylomicron TG are a rich potential source of energy Of interest, then, is the extent to which under physiological conditions they are directly extracted and utilized by tissues with a high energy demand such as heart muscle and exercising skeletal muscle. The oxidative metabolism of the heart is under most conditions covered to an extent of about two-thirds by the oxidation of fatty acids (17). In studies where arterial–coronary sinus differences for chylomicrons were meas ured nephelometrically and at the same time TG differences chemically we could further show that substantial amounts of exogenous fat were extract ed (5)

Skeletal muscle also utilizes fatty acids although these contribute less to oxidative metabolism than in heart muscle (12 14). However the ex tent to which skeletal muscle can take up exogenous TG has not been studied in man under physiological conditions The aim of this study was therefore to determine the possible extrac tion of exogenous TG in resting and xercising human skeletal muscle The forearm musculature was chosen since it offers the possibility to sample blood from the deep venous system, which almost exclusively drains muscle tissue. The forearm also contains subcutaneous tissue. The subcutaneous tissue contains large proportion of fat, which has a potential capacity of chylomicron removal As it is possible also to sample blood from veins which predominantly drain these tis sues the simultaneous removal of exogenous plas ma TG in subcutaneous tissue was also studied. Exogenous TG was given to the subjects in the

Table II *Arterial concentrations (a) and concentration differences of exogenous plasma TG (µmol/l) determined by nephelometry between artery and deep vein (a–dv) (means ± S.E.M.)*

Subj. no		a–dv	% of a		a–d	% of a
Rest	20 min			30 min		
1	1 622±15	327±20***	20.2	1 561±15	190±20***	12..
2	986±6	173±10*	17.5	1 020±3	97±5**	9.5
3	1 241±5	109±8**	8.8	1 322±4	166±7***	12.6
4	1 487±12	30±13	2.0	1 678±4	84±12***	5 0
5	673±7	135±8***	21.2	643±5	140±6	21.8
6	1 881±13	60±20*	3.2	2 088±20	96±24**	4.6
7	1 390±11	20±15^{NS}	1 4	1 514±6	62±9***	4 1
X±S.E.M.		122±40	10.6±3.3		119±18***	10 0±2.4
Exercise	10 min			15 min		
1	1 655±19	5±40^{NS}	0.3	1 613±13	−66±15**	−4.1
2	1 088±3	13±6	1.3	1 012±12	33±8***	3 4
3	1 349±4	−16±6	−1.2	1 339±3	−57±6	−4.3
4	1 933±7	−91±13	−4 7	2 031±11	−57±18***	−2.8
5	635±7	18±8	2.9	630±5	31±8**	5 0
6	4 380±16	−62±25	−2.6	2 490±16	31±25^{NS}	1 4
7	1 622±7	−33±13	−2.0	1 639±5	58±8***	−3 5
X±S.E.M.		−23±15^{NS}	−0 9±1 0^{NS}		−20±19^{NS}	−0.7±1.5^{NS}

NS=not significant, $p<0.05$ $p<0.01$ *** $p<0.001$
The statistical evaluation is based on the test of difference between means.

DISCUSSION

Methodology In this study the arteriovenous concentration differences of a fat emulsion have been determined by nephelometry which measures plasma turbidity There are however several reasons to believe that such a method also measures quantitatively the exogenous plasma TG concentration difference across the vascular bed. In earlier studies comparisons have been made between nephelometry and a chemical method and showed a high degree of agreement between the methods (5 18) No systematic arteriovenous concentration differences of ^{131}I-albumin were found In some samples however significant differences were observed and the arteriovenous differences of fat particles were corrected accordingly However the results of the study are not influenced by these corrections.

Table III *Arterial concentrations (a) and concentration differences of exogenous plasma TG (µmol/l) determined by nephelometry between artery and superficial vein (a–sv) (means ± S.E.M.)*

Subj. no	a	a–sv	% of a	a	a–sv	% of
Rest	20 min			30 min		
1	1 622±15	202±10***	12.5	1 561±15	86±9***	5.5
2	986±6	60±7*	6.0	1 020±3	80±7**	7 7
3	1 241±5	45±9***	3.6	1 322±4	97±6**	7.3
4	1 487±12	50±11	3 4	1 678±4	68±13	4 1
5	673±7	93±6**	6.4	643±5	18±7**	3.0
6	1 881±15	51±18**	2.8	2 088±20	127±22***	6 1
7	1 390±11	–	–	1 514±6		
X±S.E.M.		84±25	5.8±1.5		79±15	5 6±0.7**

Statistical symbols as in Table II.

Extraction of oxygen and lactate The oxygen extraction at rest in the forearm musculature and superficial tissue as well as the oxygen extraction and lactate release in the submaximally working forearm are in agreement with earlier data from healthy young subjects (15 24). The far lower a–v difference for oxygen in subcutaneous tissue than in muscle signifies a far greater blood flow in relation to oxidative metabolic rate a fact of importance when the extraction of TG in the tissues is discussed

Removal of exogenous plasma TG at rest At rest there was a significant removal of Intralipid® both at 20 and 30 min in the skeletal muscle of the forearm. This removal corresponded to about 10% of the arterial concentration of fat particles During the same time the subcutaneous tissues of the forearm removed about 6% of the arterial concentration of fat particles. The smaller fractional removal in subcutaneous tissue might be due to the greater blood flow in this tissue in relation to its metabolic demand Similar extraction figures for the splanchnic region have recently been found in a parallel study in which an arterio-portal extraction of 5.3% of the arterial concentration of fat particles was demonstrated during the same period (11). In an earlier study on myocardial removal of exogenous plasma TG the corresponding removal figure was 6% although this figure was the mean obtained from samplings taken at 1 2, 3 and 4 hours during an infusion of Intralipid® From the present short term study it is not possible to conclude whether the skeletal muscle removal represents a steady uptake which can be maintained for a longer time.

Removal of exogenous plasma TG during work. No mean arteriovenous concentration difference of fat particles was observed during work. An explanation of this finding might be that the maximal rate of removal is utilized already at rest, with a resulting marked decrease in a–v concentration difference when the forearm blood flow is increased during work, presumably about five times (24). Support for the conception that skeletal muscle cannot increase its uptake of exogenous TG during work is found in a study in which Intralipid® was given as a constant rate i.v. infusion over 4 hours and the steady aterial concentration of exogenous TG was not influenced when the subjects started moderately heavy bicycle work after 2 hours (6). However an alternative explanation might be that the fat particles removed are not taken up by the muscle tissue but merely adhered to the vessel walls At the onset of forearm work they might then be washed out again maybe by the mechanical effect of the muscle contractions The finding of some both positive and negative significant a–d concentration differences during work might to a certain extent support the interpretation that the fat particles are not taken up but loosely attached to the vessel walls.

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Table I *Plasma triglycerides (TG) and cholesterol (CHOL) body fat (BF) fat cell size (FCS) and k-value for intravenous glucose tolerance (IVGTT) and per cent of linoleic (18 2) linolenic (18 3) and arachidonic (20 4) acid in the extracted adipose tissue lipids in male subjects with different types of hyperlipoproteinemia*

	Types of hyperlipoproteinemia					
	N	II A	II B	III	IV	V
n	14	3	2	4	18	2
TG (mmol/l)	1 46±0.47	1 65±0 63	3.86±2.09	5 75±4.58	4.37±1 96	13 43±4 98
CHOL (mg/100 ml)	220±37	351±29	317±27	358±137	272±33	533±49
BF (kg)	11.9±5 7	9 1±1 6	8.5±0.3	14.9±4.2	18.1±4 9**	16.4±0.1
FCS (μ)	75±13	84±4	85±18	87±15	88±5	85±1
IVGTT (k-value)	1.86±0 85	1.63±0 74	0 72±0 48	1 06±0.24	1.21±0.57*	1 10±0.01
18 2	11 77±..47	11 78±1 42	13.90±0.60	13 17±3 62	11 10±..12	10.22±0 31
18 3	..30±0 78	1 97±0.77	1.06±0 07	1 70±0 64	1.32±0 47***	1.08±0.1**
20 4	0.97±0.50	0 60±0.58	0.08±0.12	0 40±0.30*	0.37±0.22*	0.23±0.02

According to the WHO classification, N=normal.
** indicate that the difference in relation to group N was significant with a p-value of 0.05 0.01 and 0 001 (Student s t-test)

than normals. The content of 18 3 and 20 4 was low in the groups with HTG while that of 18 2 was the same in all groups On the other hand subjects with hypercholesterolemia but normal plasma TG levels did not differ from the normals.

The correlation coefficients between the metabolic and morphologic parameters are given in Table II The content of 18 3 and 20 4 correlated *positively* to the k value but *negatively* to plasma TG levels Body fat and fat cell size were not significantly correlated to the k value but negatively the content of 18 3 and 20 4

As there may be multiple interactions between all ese parameters, the relationships have been further analysed by partial correlation analysis Table III reveals that the correlation coefficient between the k value and 18 3 is only slightly changed from 0.59 to 0.51 when plasma TG are kept constant, and that between plasma TG and 18 3 goes from –0.55 to –0 44 when the k-value is kept constant. When the influence not only of plasma TG but also of fat cell size and body fat was eliminated the correlation between the k value and 18 3 was still approximately the same (Table III).

When the association between the k value and the TG concentration was analysed by calculation of partial correlation coefficients (Table IV), it was found that this association remained when fat cell size or body fat was kept constant but disappeared when 18 3 was kept constant.

DISCUSSION

The results thus show that with decreasing percentage of 18 3 in AT the intravenous glucose tolerance declined and the plasma triglyceride concentration increased These two associations with

Table II *Correlation coefficients between plasma TG the k-value for the IVGTT and different adipose tissue characteristics*

Abbreviations, see Table I

	BF	μ	log TG	18 3	20 4
log k	– 17	– 13	–.36	.59***	47*
BF		.59*	.53***	– 45	– 46**
μ			49***	– 43**	–.54 **
log TG				–.55**	–.55***
18 3					.85***
20 4					

p<.05 p<.01 *** p< 001

Table III *Relation between 18 3 and the k alue for the IVGTT and plasma TG concentration (partial correlation coefficients)*

Abbreviations, see Table I

Parameters correlated		Parameters kept constant	-value
log k	18 3	log TG	51
log TG	18 3	log k	−.44
log k	18 3	log TG FCS BF	.54

18 3 were largely independent of each other The usual relation between GI and HTG (2) however appeared to be due to their common association with the content of 18 3 This suggests that the high frequency of GI found in HTG might be related to a low content of 18 3 in AT associated with both HTG and GI It has indeed been suggested that the association between GI and HTG may be mediated through a common association to the same factor perhaps adiposity (2) Since the correlation between plasma TG and the *k* value was not diminished appreciably when partial correlation was performed and body fat was kept constant, our results rather suggest that the common factor is AT 18 3 which in fact was related to adiposity (Table II)

The present relations were found for the percentage of 18 3 in AT Since there was no significant relation between the *k* value and body fat or fat cell size and since the correlation coefficient was unaffected when the influence of body fat was eliminated it is unlikely that the relation between the *k*-value and 18 3 was due to changes in body fat.

Similar relations to those for 18 3 were also found for 20 4 It should be stressed that these acids have only been identified tentatively by retention time factors.

Table IV *Relation between the k value for the IVGTT and plasma TG concentration (partial correlation coefficients)*

Abbreviations, see Table I

Parameters correlated		Parameters kept constant	*r*-value
k	log TG		−.36
k	log TG	FCS	− 35
k	log TG	BF	−.32
k	log TG	18 3	− 05

In earlier studies some abnormalities have been found in the fatty acid composition of various plasma lipid fractions in different types of hyperlipidemia or in atherosclerosis the most common finding being a decrease in 18 2. Schrade et al (10) thus observed a negative correlation between the percentage of 18 2 and 20 4 and total plasma lipids Similarly Kingsbury et al (8) found lower 18 2 and 20 4 in subcutaneous AT in subjects with atherosclerotic diseases than in a control material It is difficult to connect these findings with our finding of normal 18 2 in AT Hagenfeldt et al (7) recently reported a low content of 18 3 and 20 4 in the plasma free fatty acids (FFA) in patients having survived a myocardial infarction. Since such patients frequently have either HTG or GI, it is possible that their AT content of 18 3 and 20 4 was low which might cause a low FFA content of these acids since the majority of plasma FFA are mobilized from AT

Both 18 3 and 20 4 can be derived from 18 2 while 18 2 cannot be synthesized in the body but must be provided in the diet Since the content of 18 2 in AT was normal it seems likely that the low content of 18 3 and 20 4 is due rather to changed metabolism than to a dietary deficiency Of interest in this connection is the finding in diabetic animals that there is an impaired synthesis of arachidonic acid from linoleic acid (6)

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POLYMORPHIC ACETYLATION OF PROCAINE AMIDE IN HEALTHY SUBJECTS

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Abstract The acetylation of procaine amide has been studied by means of gas liquid chromatography in 33 healthy human volunteers. The acetylator phenotype was determined by measuring unchanged and acetylated sulphapyridine in urine. Slow acetylators of sulphapyridine excreted significantly less procaine amide in acetylated form in the urine than rapid acetylators (9±1% against 19±4%). Hence it is suggested that the acetylation of procaine amide is subject to the same genetic polymorphism as that of isoniazid and some sulfonamides.

Procaine amide has been shown to be an effective drug in the treatment of ventricular arrhythmias (7 8, 12) but has the disadvantage of inducing a systemic lupus erythematosus (SLE)-like syndrome in some patients during long-term treatment (1)

A similar syndrome can also be induced by hydralazine which like isoniazid and some sulfonamides has been shown to be polymorphically acetylated in man (6) Since this SLE-like syndrome occurs predominantly in the phenotype slow acetylators (14) it was of interest to explore the possibility that also procaine amide is acetylated polymorphically in man Indirect evidence for this had already been obtained by investigating the biotransformation of procaine amide in cardiac patients (9 10) However these patients were not drug-free and some of them also had renal impairment, which complicated interpretation of the results. Therefore we thought it important to study procaine amide acetylation in healthy subjects who on a separate occasion underwent a sulphapyridine phenotyping test.

MATERIAL AND METHODS

Thirty-three healthy and drug-free volunteers, 6 females and 27 males, 23–46 years old (mean 26), participated in the study Their state of health was determined by history physical examination, ECG and routine laboratory tests including Hb ESR, serum creatinine and serum levels of bilirubin alkaline phosphatases, GOT GPT and LDH activities All subjects gave their informed consent

The subjects were first phenotyped as slow or fast acetylators of sulphapyridine in the following way The fasting subjects were given 500 mg sulphapyridine (as commercial Septipulmon tablets supplied by AB Pharmacia, Uppsala, Sweden) in the morning and food intake was not allowed until at least hours later Urine specimens from the 7th–8th hour after the oral intake of sulphapyridine were collected and analysed within 1 day according to Schroder and Evans (16).

At least 1 week later the procedure was repeated with procaine amide. The fasting subjects were given 500 mg procaine amide hydrochloride orally as the commercial tablets of 250 mg (Pronestyl® Squibb). One blood sample was drawn just before the dose and used as plasma blank. The other blood samples were drawn 1 2.5 5 and 7 hours after the dose in order t verify adequate absorption of the drug. The concentration of procaine amide in plasma was determined by the spectrophotofluorometric method described by Koch-Weser and Klein (11) but substituting benzene for toluene Plasma samples were stored at −20°C for up t 1 month before analysis

Urine was collected for 8 hours at 4 intervals (0–1 1–4 4–6, and 6–8 hours after drug ingestion). Urine samples were stored at +4°C for up t 2 weeks before analysis of procaine amide and N-acetylprocaine amide

The method used for analysis of procaine amide and N-acetylprocaine amide has been described in detail in previous paper (10) Because of the lower concentration predicted in this investigation compared to that in cardiac patients, standard urine samples containing 80 250 500 and 1600 μg/ml procaine amide and 20, 60 160, and 320 μg/ml N-acetylprocaine amide were analysed in every assay procedure for specimens from volunteer subjects W found it also suitable to inject 1 0 μl sample into the gas chromatograph for the same reason A 5% OV17 chromatographic column prepared in the same manner as the column sed for plasma analysis in our earlier study was sed for analysis. All determinations were performed in duplicate together with urinary blanks.

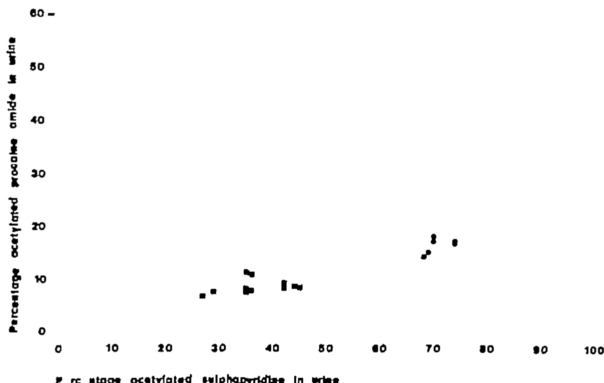


Fig. 1. Relationship between percentage of acetylated sulphapyridine in urine and urinary N-acetylprocaine amide in % of the sum of excreted procaine amide and N-acetylprocaine amide. Rapid (●) and slow (■) acetylators according to sulphapyridine test ($n=33$).

Statistical methods

The significance of differences between means was tested by Student's *t*-test. The significance of correlations between acetylated sulphapyridine and procaine amide was tested using Spearman's rank correlation (17).

RESULTS

Rapid and slow acetylator phenotypes were clearly defined by means of the sulphapyridine test (Fig. 1). The mean percentage of acetylated sulphapyridine in the urine of slow acetylators was 37 ±6% (mean ±S.D.) and in the urine of rapid acetylators 72 ±4% (mean ±S.D.). Seventeen of 33 subjects (51.5%) were slow acetylators, which is within the same range as reported by Schröder and Evans (16) when investigating volunteers.

In fast acetylators of sulphapyridine the urinary excretion of N-acetylprocaine amide accounted for 19±4% (mean ±S.D.) of the total sum of recovered procaine amide and N-acetylprocaine amide in the urinary specimen collected during 1–4 hours after administered dose. The corresponding percentage in the slow acetylator group was 9±1% (mean ±S.D.). The differences between the group means reached statistical significance ($p<0.001$).

A significant correlation was found between the percentage acetylation of sulphapyridine and procaine amide in urine ($r=0.80$; $p<0.001$, Spearman's rank correlation). Table I gives the urinary excretion of acetylated procaine amide in all the different urine portions for slow and rapid acetylators. In a frequency distribution histogram the best separation of groups was obtained with the data from the urine collected 1–4 hours after administered dose.

Table I. *Percentage acetylation of procaine amide in the different urine portions with comparison between slow ($n=17$) and rapid ($n=16$) acetylator phenotypes determined by means of sulphapyridine (mean ±S.D.)*

Hours after procaine amide ingestion	Slow acetylators	Rapid acetylators
0–1	4.0±1.0	8.2±2.0
1–4	8.7±1.3	18.9±4.0
4–6	14.9±2.2	31.2±6.1
6–8	21.8±3.2	41.5±7.6
0–8	10.6±1.6	22.5±4.7

Table II *Plasma concentration and plasma half life of procaine amide in slow and fast acetylators (mean±S.D.)*

	Hours after dose	Plasma concentration of procaine amide (μg/ml)	Plasma half life of procaine amide (min)
Fast (n=16)	1	2.2±0.5	176±33
	2.5	1.6±0.2	
	5	0.8±0.2	
	7	0.5±0.1	
Slow (n=17)	1	2.3±0.4	180±38
	2.5	1.5±0.5	
	5	0.8±0.3	
	7	0.6±0.1	

The mean plasma concentration of procaine amide did not differ between slow and fast sulphapyridine acetylators nor did the plasma half-life of procaine amide (Table II).

DISCUSSION

The incidence of slow acetylators in our material was about 50% which is within the range reported by Schröder and Evans (16). Conclusive evidence has been presented that the acetylation of procaine amide follows the same pattern as that of sulphapyridine. There is a significant intraindividual correlation between the acetylation rate of the two drugs showing that procaine amide can be added to the list of drugs whose acetylation is governed by the same genetic polymorphism.

There was no difference between rapid and slow acetylators regarding either the initial (or mean) plasma concentration or the plasma half-life of unchanged procaine amide. This is probably explained by the fact that procaine amide is excreted unchanged in the urine to a great extent. It has recently been suggested that hydralazine is acetylated during the first passage through the intestine or liver explaining the lower peak levels but identical half lives of the drug in fast than in slow acetylators (2, 15). Evidently this mechanism does not play a major role for procaine amide.

N-acetylprocaine amide is a potentially active metabolite (4, 5, 9) that accumulates in renal failure. Preliminary data show that the relation between N-acetylprocaine amide and unchanged procaine amide in plasma is 3:2 in patients with impaired renal function whilst in patients with normal kidney function the ratio is the reverse (10). The degree of accumulation of N-acetylprocaine amide can be expected to differ between the two phenotypes in renal failure. Phenotyping such patients may thus be of clinical value.

Among the drugs which have been proven to induce an SLE-like syndrome procaine amide, hydralazine and isoniazid seem to feature most frequently in published reports (13). Slow acetylators have been shown to be more prone than rapid ones to develop various side-effects after the latter two drugs, e.g. polyneuritis after isoniazid (3) and the SLE-like syndrome after hydralazine (14). It will therefore be of considerable interest to determine whether a similar relationship exists for procaine amide too. Further studies with determination of the phenotype in patients who have developed a procaine amide-induced SLE-like syndrome are needed to answer this question.

ACKNOWLEDGEMENT

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ACETYLATOR PHENOTYPE AND THE ANTIHYPERTENSIVE RESPONSE TO HYDRALAZINE

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Abstract Twenty-three out-patients with mild or moderate essential hypertension hav been treated with combination of hydralazine (37.5–150 mg daily) and oxprenolol (60 mg daily). Before treatment the patients were phenotyped for polymorphic acetylation by means of the sulphamethazine test 12 proved to be low and 11 rapid acetylators. A significant correlation was found between daily doses of hydralazine and the plasma hydralazine levels, separately in slow (r=0.480) and in rapid (r=0.580) acetylators. The antihypertensive response to hydralazine correlated well to plasma hydralazine levels. The mean fall of BP in slow acetylators was 33/23 mmHg in supine and 20/18 mmHg i standing position The corresponding values in rapid acetylators were 22/15 and 21/15 mmHg The average daily doses of hydralazine needed for these responses were 1.3 mg/kg in slow and 1.6 mg/kg in rapid acetylators. T reduce the systolic BP by 20 mmHg, 1 0 mg/kg of hydralazine was needed in slow acetylators rapid acetylators needed significantly higher dose of 1 4 mg/kg. During a follow-up of 1 year there have been virtually no side-effects The results tally with the previous finding of Zacest and Koch-Weser who demonstrated a similar correlation during the triple-drug regimen. It seems as if hypertensive patients can be successfully treated with hydralazine and β-blocking drugs without knowledge of the patient acetylator phenotype. However acetylator status is determinant of tissue levels and long-term toxicity of hydralazine and patients should be phenotyped because β-blockers may mask the warning side-effects.

Like isoniazid and sulphamethazine (SMZ) hydralazine (HZ) is acetylated on th polymorphic pattern (2) The polymorphism of acetylation correlates to the activity of liver enzyme acetyltransferase the slow acetylator phenotype s an autosomal recessive trait and there are racial differences with regard to its frequency (2). Slow acetylators are liable to generate antinuclear antibodies or systemic lupus during long-term treatment with HZ (9) This is probably due to an accumulation of HZ in tissues since Zacest and Koch-Weser (13) have shown that the same dose given to slow acetylators results in higher plasma HZ levels than in rapid acetylators.

It is known that the antihypertensive response to HZ in rats is related to its concentration in blood and aortic wall (6) We initiated the present trial to correlate the acetylator status and plasma HZ levels of hypertensive patients to their antihypertensive response to HZ. During our trial Zacest and Koch-Weser (13) reported that this correlation exists in situation where HZ is administered in combination with propranolol and diuretics

PATIENTS AND METHODS

Patients

Twenty-three out-patients, 12 males and 11 females, with established essential hypertension, were admitted to the trial. *Their ages ranged from 24 to 58 years (mean 46)* and their weights from 61 to 118 kg (mean 80) The patients were accepted for the trial if their diastolic BP measured during three subsequent visits to the Out-patient Clinic at 1-week intervals, was consistently 95 mmHg or more. All cases with history of angina pectoris, cardiac decompensation, asthma or autoimmune diseases were excluded. After fulfilling these criteria the following tests were obtained in all patients, complete blood count, ESR, serum electrolytes, creatinine and uric acid, urinalysis, 24-hour urinary excretion of metanephrines LE cells, rheumatoid factor and antinuclear antibodies ECG chest X-ray pyelography and examination of ocular fundi On these ground the patients were classified to fulfil the criteria of grades I–II hypertension (WHO)

Treatment of blood pressure

BP was measured throughout the tudy in the
of the Out-patient Clinic by the same trained

the same standard mercury sphygmomanometer Three to six measurements at 5-min intervals in supine and 2 3 measurements in standing position were taken between 4 and 6 p.m. and the three lowest values were averaged. Heart rate was counted from the radial pulse after the last BP recording in each position. Patients were checked in the Out-patient Clinic at weekly intervals. At each visit they were questioned about side-effects and their general condition, BP and heart rate were measured and tablets were counted. At the end of the trial, which lasted 3 months and one year after starting the treatment, blood count, ESR urinalysis, serum creatinine LE cells and antinuclear antibodies were investigated After the three visits during which the baseline BP was recorded, treatment was started with HZ at a dose of 12.5 mg three times daily

Since our previous experience showed that treatment with HZ alone up to 150 mg daily resulted in therapeutic failure and intolerable side-effects, the treatment with a β-adrenoceptor blocking drug oxprenolol (20 mg t.i d) was combined with the HZ treatment from the very beginning. The dose of HZ was increased at 1-week intervals by 37.5 mg up to a total daily dose of 150 mg or until the patients were normotensive or until intolerable side-effects occurred

Chemical measurement of drugs

The polymorphic acetylator phenotype of the patients was determined by using SMZ as test drug since it is acetylated like isoniazid and HZ (2) and easy to measure. SMZ tablets (10 mg/kg) were given to the patients during the first visit to the Out-patient Clinic. In the morning of the day of their second visit they took a sample of morning rine for a reference ingested the tablets with a light breakfast, and the 6-hour urine sample was taken in the Out-patient Clinic. The aliquots of urine were stored at −20°C for several weeks before SMZ and acetyl SMZ were assayed by the chemical Bratton-Marshall procedure (12) This simple measurement of unacetylated and acetylated SMZ in urine is accurate enough to separate slow and rapid acetylators (7)

To estimate the plasma HZ, samples of venous blood were drawn during the visits 2–4 hours following the last dose of HZ. Plasma was separated by centrifugation, acidified by adding two *drops of 10 N hydrochloric acid*, and kept at −20°C for several weeks ntil assayed according to the spectrophotometric method (11) which in our hands proved reliable in the concentration range of 0 06–4 µg/ml

Statistical analysis

Student s t test was used for the statistical analysi of the results.

RESULTS

Acetylator phenotype and plasma HZ levels On the basis of present results and our previous experience (3) 12 patients of a total of 23 were classified as slow acetylators, their ratios of unacetylated SMZ/total SMZ in urine ranging from 29 to 52% The respective ratios in 11 rapid acetylators ranged from 6 to 19%

The plasma HZ levels related to the HZ dosage are illustrated in Fig. 1 Generally the plasma levels were higher with increasing doses but the highest levels were not reached in rapid acetylators. When the plasma HZ levels are plotted against the HZ dosage separately in either phenotype the correla-

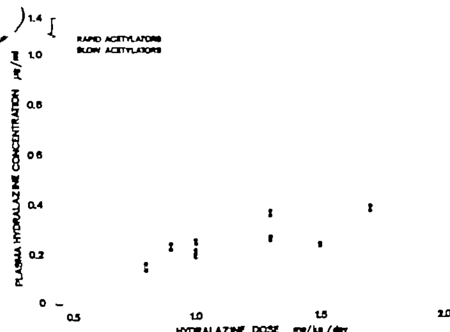


Fig 1 Correlation between daily hydralazine dose and plasma level of the drug in slow (r=0.480) and in rapid (r=0 580) acetylators

Table I *Effect of combined hydrala.ine+ox prenolol on blood pressure (mmHg mean ±S.E.) in slow and rapid acetyl tors*

	Supine BP		Standing BP	
	Systolic	Diastolic	Systolic	Diastolic
Slow acetylators				
Before treatment	176±6	114±4	164±7	115±4
During treatment	143±3	91±2	144±5	97±3
Rapid acetylators				
Before treatment	167±5	110±1	166±8	112±2
During treatment	145±4	95±3	145±5	97±3

tions are statistically significant ($p<0.05$) in both instances

Since the homozygous or heterozygous acetylator genotype can be revealed by special technique, and the amount of polymorphic liver acetyl transferase is consequently known to be higher in homozygous than in heterozygous rapid acetylators, the plasma HZ levels achieved within a narrow dose range 1.2–1 6 mg/kg were plotted against the SMZ acetylation ratio unacetylated SMZ/total SMZ. It appeared that high HZ levels (≥0.5 µg/ml) were measured in slow acetylators with an SMZ ratio above 35% whereas all rapid acetylators and some slow acetylators exhibited lower HZ levels.

Antihypertensive respons to HZ The dose of oxprenolol used (20 mg t.i.d.) which is far below its actual antihypertensive dose range (4) proved effective in preventing the tachycardia and headache that are associated with moderate doses of HZ alone. The antihypertensive response of the patients to treatment is seen in Table I There was evidently a satisfactory clinical effect yet the patients remained on the borderline hypertensive level To obtain these responses in slow acetylators an average HZ dose of 1.3 mg/kg was needed compared with 1 6 mg/kg in rapid acetylators The difference s not significant.

The verage reduction of supine diastolic pressure in slow acetylators (23 mmHg) was significantly more ($p<0.05$) than in rapid acetylators (15 mmHg) This indicates that slow acetylators were slightly more responsive to given dose of HZ. In order to reduce supine systolic BP by 20 mmHg or more, 1 0 mg/kg HZ was needed in slow acetylators and 1 4 mg/kg in rapid This difference is statistically significant ($p<0.05$)

Fig. 2 illustrates the correlation between the antihypertensive response and plasma HZ levels, with the data for slow and rapid acetylators combined The response improves with increasing

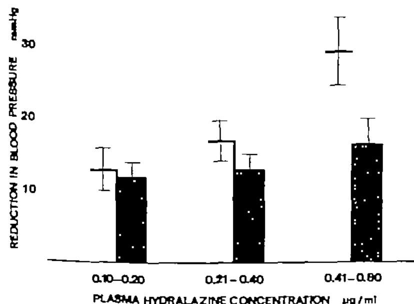


Fig. 2 Effect of plasma hydralazine concentration on the antihypertensive response in all patients studied. Open columns=systolic, stippled columns=diastolic BP Means±S.E.

Table III *24-hour uri ary excretion of trace elements (μg) with no known biological function before and during treatment with hydrala ine*

	Ag		As		Au		Cs	
Case no	Before	During	Before	During	Before	During	Before	During
1	0 46	0.32	42	210	0.00035		14	11
	0 43	1.6	610	510	0.0021	0.0044	10	13
3	0.37	0.52	100	67	0 066	0 019	15	17
4	0 41	0.58	85	66	0 00072		8 9	15
5	0 47	0 28	110	86	0 00087	0 00087		7 0
Mean±S.D	0 43±0.04	0 66±0 54	190±240	190±190	0 014±0 029	0 0081±0 0096	12±3 0	13±3 8
Mean difference	+0.23		−2.2		−0 016		+2.0	

that hydralazine disease may be related to a metal deficiency (2 10)

It has been reported that hypertensive patients excrete large amounts of cadmium and manganese in the urine and that treatment with hydralazine *lowers the urinary excretion of these elements (6)* However in a previous study I found that the urinary excretion of 17 trace elements in untreated hypertensive patients did not differ significantly from that in normotensive subjects (11)

The aim of the present study was to investigate the urinary excretion of antimony arsenic bromine, cadmium cesium, cobalt, copper gold iron mercury molybdenum, rubidium, scandium selenium silver tungsten and zinc in hypertensive tients before and during treatment with ydralazine

MATERIAL AND METHODS

Five male hypertensive patients ranging in age from 29 to 46 years, were subjected to the study Three patients had mild hypertension with diastolic BP on admission varying between 105 and 115 mmHg The other two patients (nos and 3) had more severe hypertension with diastolic BP on admission of 150 and 125 mmHg, respectively Patient 2 also had an enlargement of the heart (870 cm³/m²) and this patient and one of the others (no. 5) had slight proteinuria. The patients had not previously received any antihypertensive medication They were treated with hydralazine (Apresolin® 75 mg/day) and five 4-hour urine samples were pooled and 1 ml sample of the pooled ne was pipetted into quartz ampoule for trace element analysis In a previous study (11) urine from untreated patients was analysed in the same manner

The trace element determinations were performed with neutron activation analysis. The neutron flux used w s 2 10¹³ n/cm²/sec and the samples, together with standards, were irradiated for 4 hours. The chemical separation technique ha been described elsewhere (7 12).

Statistical methods

The tatistical analysis was ndertaken with the paired *t*-test. The degrees of significance have been expressed as follows: *—not significant,* 0 05<*p* *almost significant,* 0 01<*p*<0 05 significant, 0 001<*p*<0 01 highly significant, *p*<0 001

RESULTS

The urinary excretion of the different trace elements studied before and during treatment with hydralazine is presented in Tables I–III Mean values S D and paired mean differences have been calculated The amounts excreted are given in *μg/24 hours. In these Tables the trace elements* have been divided into those with known (Table I) suspected (Table II) and no known biological function (Table III)

Among the trace elements with known biological function, it was noted that the mean amount of copper excreted during treatment with hydralazine was more than twice that before treatment the paired mean difference of 89 *μg*/24 hours was almost significant. On the other hand the urinary excretion of zinc was almost significantly lower during treatment compared to before. The excretion of cobalt and iron did not change significantly

The paired mean differences for all five trace elements with suspected biological function were very small and insignificant among the trace elements with no known biological function no significant differences were observed between the urinary excretion during and before treatment The results expressed in *μg*/g excreted creatinine per 4 hours gave the same statistical correlations.

Hg		Sb		Sc		W	
Before	During	Before	During	Before	During	Before	During
0 66	0 45	1 7	2.0	0 070	0.22	41	5.6
1.8	1.3	2.6	1 9	0 024	0 014	12	5 6
2 4		2.4	1 4	0 016	0.038	16	16
2 4	2.7	1.3	2.0		0.021	6 5	4.9
1.4	1.9	2.0	0.67	0 0078	0 0064	3 0	1.3
1 7±0 73	1.6±0.95	2.0±0 57	1 6±0 57	0.029±0.028	0 060±0 090	16±15	6.3±5 7
−0 002		−0.41		+0 014		−9 4	

DISCUSSION

The most prominent finding in this study is the increased urinary excretion of copper during treatment with hydralazine. The mean value before treatment (77 μg/24 h) is somewhat higher than the mean found previously (50 μg/24 h) in a larger group of untreated hypertensive patients (11) This is probably due to the fact that two of the patients in the present study had slight proteinuria, since copper excretion is elevated in this disorder (1 4) However the increase in this excretion during treatment with hydralazine cannot be explained by proteinuria as three of the patients did not have this disorder and in the two who did the proteinuria did not increase during the treatment.

It has also been reported that zinc excretion is increased in patients with proteinuria (3) which explains the somewhat higher mean value for the untreated patients in the present study as compared to that obtained previously in a larger group of untreated hypertensive patients (11)

The clinical significance of the increased urinary copper excretion during treatment with hydralazine is difficult to interpret. It is not known whether the increase is a temporary phenomenon or whether it persists during treatment for longer periods, neither has the possibility been studied that copper concentrations decrease in serum or tissues

ACKNOWLEDGEMENT

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FEVER AND HAEMOLYSIS IN HODGKIN S DISEASE

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Abstract. One hundred and four patients with Hodgkin disease have been studied retrospectively in order to evaluate the relationship between fever associated with the disease (Pel-Ebstein type) and the development of anaemia. In the material 19 episodes of fever were found to be of this type. The mean loss of Hb during fever period was 14% (range 33) From the rate of decrease in Hb it was deduced that this was at least partly caused by an increased destruction of erythrocytes. There was a significant correlation between the thermal xposure (expressed either as the duration of fever the maximum body temperature during the fever period or the sum of the temperature maxima) and the degree of erythrocyte loss. The loss of Hb was self-limited in spite of persistent fever Furthermore, there seemed to be an inverse relationship between the degree of preexisting anaemia and the fever induced relative loss of Hb. A possible explanation is that the older part of the erythrocyte population is more sensitive to the effect of fever

Haemolytic episodes in conjunction with fever of the Pel-Ebstein type in Hodgkin s disease have been described *in a few cases (4 11) From in*vestigations in rabbits it has been suggested that elevation of the body temperature within the fever range induces an increased destruction of erythrocytes (5 7) Furthermore in vitro studies have shown that human erythrocytes are also sensitive to destruction by small rises in temperature (8 9).

The aim of the present study was to examine a possible general relationship between the elevation of body temperature during Pel-Ebstein fever and erythrocyte destruction in patients with Hodgkin disease

MATERIAL AND METHODS

Altogether 104 cases of Hodgkin disease confirmed by biopsy admitted to the department in the period 1969–73 were studied retrospectively. In 30 of the patients febrile episodes were observed 9 patients were excluded from this material because of difficulties in interpreting changes in the Hb level (bleeding, transfusions cytotoxic treatment). The remaining 1 patients had total of 26 episodes of fever In 19 of these episodes there were no signs of *infection as judged by clinical examination*, bacteriological studies of blood and urine, and chest X-ray and consequently the fever was considered to be related to the disease itself. I most cases the fever curve was of the Pel-Ebstein type In 6 cases the febrile episode was caused by pneumonia and in 1 case by acute cystitis.

Eighteen of the patients were in stages III or IV and one patient in stage II (Paris-Rye classification) One patient had splenomegaly two had hepatomegaly and in one patient splenectomy had been performed years before.

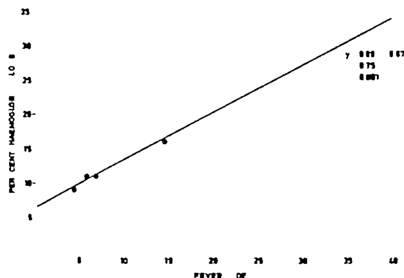
Only elevations in temperature above 37.5°C for more than one day were registered, and all calculations were based upon the maximum value for each day of fever The degree of heat xposure was expressed as 1) the duration of the fever period 2) the highest temperature measured during the fever period or 3) the sum of the daily maximum values throughout the fever period (fever index).

The haematological consequences of an attack of fever were estimated from changes in the Hb concentration and the reticulocyte counts The changes in Hb were expressed as th loss (%) during the fever period. *Major bleeding* was excluded on clinical grounds. In all cases the direct Coombs test was negative

RESULTS

During the 19 episodes of fever the mean decrease in the Hb concentration was 14% (range –33) In order to examine the mechanism of the decrease in Hb the results from the early part of the fever period were analysed in detail. The mean decrease during the first week of fever was 11% with an average of 1 7%/day (range 0 6–3.2) During the same period the mean reticulocyte count was 0.9% (range 0.4–.0)

Fig 1 shows the relationship between the duration of fever and the loss of Hb as percentage of the prefebrile Hb concentration. Although the results have a rather wide scatter regression analysis

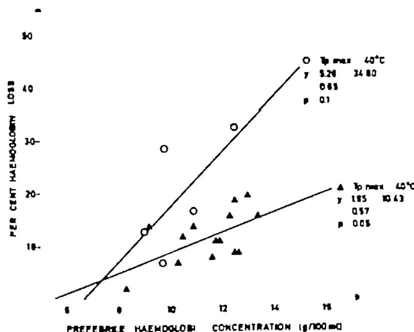


F g 3 Relationship between fever inde and loss of Hb

plained by a decreasing effect on the RBC with increasing fever index

The results i the patients with hepatosplenomegaly and the patient who previously had a splenectomy did not differ significantly from the other part of the material.

In Fig. 4 the loss of Hb is related to the prefebrile Hb concentration in each fever period. The patients are divided into two groups according to their maximum fe er level In the low temperature group (<40°C) there was a signif cant ncrease ($p<0.05$) in the loss of Hb with ncreasing values of prefebrile Hb Also in the high temperature group (>40°C) such a correlation seems to exist. As see Fig the curve is steeper although the result is not significant ($p>0.1$) Both regression lines intersect with the abscissa in the region of about 6 g/100 ml thus indicating a lower limit of prefebrile Hb below



F g 4 Relationship between the prefebrile Hb alue and the relative loss of Hb during fever The patients are divided into tw groups ac cording to the temperature maximum

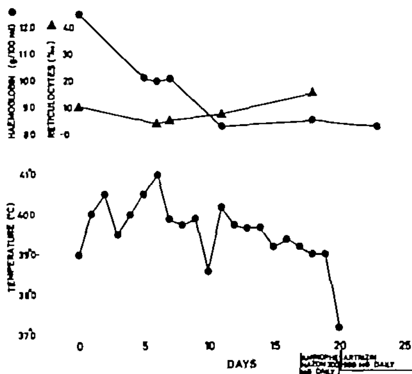


Fig 5 Course of Hb concentration and reticulocyte count in relation to a fever episode i one of the patients.

which no decrease in the Hb concentration occurs as a result of fever

Fig. 5 shows the changes in the Hb concentration during the course of a fever period and again the importance of the Hb level for the degree of erythocyte destruction is illustrated During the initial of the fever period (about 10 days) there is a ase in Hb from 12.5 to about 8.3 g/100 ml reafter in spite of continuous fever for more n 1 week, the Hb concentration seems to be constant. The stabilization of the Hb level does not seem to be due to an increase in erythropoiesis as there was no increase in the reticulocyte count. Also using the rate of decline in Hb concentration during the first 10 days of fever as a parameter of the intensity of haemolytic activity it can be calculated that the level of stabilization in the later part of the fever period (provided haemolytic activity is unchanged) should have been lower if all the red cells were equally sensitive.

Full restitution of the Hb concentration was not seen for several days after the fever had subsided indicating that the changes in Hb concentration could be only partly due to changes in the plasma volume during the fever period.

The influence of any erythropoietic response on the changes in the Hb concentration was estimated from the reticulocyte count during the fever period. There was no sign of increase in the reticulocyte count (mean 1 0% range 0.3–1 7)

DISCUSSION

This study shows a general tendency towards a decrease in Hb concentration in conjunction with episodes of fever in patients with Hodgkin s disease From the rate of decrease in Hb (1 7%/day) compared with the reticulocyte count in the initial part of the fever period it follows that this is due to increased erythrocyte destruction since major bleeding or changes in plasma volume could be excluded

The present study gives no direct information about the mechanism of the erythrocyte destruction, and several possibilities should be considered In this series the direct Coombs test was negative but this does not entirely exclude an autoimmune mechanism as discussed by Ranløv and Videbæk (11)

Another explanation could be that erythrocyte survival depends in some way directly on the body temperature The quantitative estimate of the thermal exposure used in this study based either on the duration of fever the maximum temperature meas-

ured during the fever period, or the sum of the temperature maxima, represents an obvious approximation. Both the absolute rise in body temperature and the duration of the exposure may be important but the same fever index can be obtained from either a short intensive fever period or a prolonged fever period with only a slight increase in temperature. Furthermore, if there is a maximum susceptibility of the red cell population above which no further destruction occurs any attempt to correlate the erythrocyte destruction with the exposure expressed as either the duration of fever or the fever index will give a misleading result. Nevertheless the investigation demonstrates a correlation between the degree of thermal exposure and the loss of Hb.

Experimental studies in rabbits using different methods of increasing body temperature (5 7) as well as in vitro studies (8 9) support the hypothesis that temperatures within the fever range increase the destruction of red cells. The findings of the present study may be a clinical correlate to those results.

Fig. 5 shows that a maximum erythrocyte destruction is obtained in spite of persistent fever This pattern of self-limited cell loss which was not explained by a compensatory increase in erythropoiesis, is in accordance with previous findings during experimental fever in rabbits (7) Also, the influence of the prefebrile Hb level on the relative loss during fever (Fig. 4) points to a self-limited type of erythrocyte destruction. A possible explanation is that only the older part of the erythrocyte population is susceptible to the heat exposure, as has been shown for rabbit erythrocytes (6)

To elucidate this problem different models of erythrocyte destruction have been outlined in Fig. 6. Each part of the figure represents the cell age profile of the erythrocyte population by showing the fraction of cells at various cell ages. In both column *A* and *B* three levels of total prefebrile erythrocyte mass are demonstrated viz *E* (normal state) 3/4 *E* and 1/2 *E*. Column *A* demonstrates situations with an unchanged cell age profile at different degrees of prefebrile anaemia and column *B* situations in which prefebrile anaemia is accompanied by a change in the cell age profile with decreasing number of older cells. The heat sensitive erythrocytes are symbolized by the hatched area, and, using a simplification it is assumed that all erythrocytes above a certain cell age will be destroyed by fever Irrespective of the mechanism in relation to cell age the situation in column *A* cannot explain the results seen in this study as this would give a constant relative cell loss at different levels of prefebrile Hb and no lower threshold as indicated in Fig. 4 Only the situation presented in column *B* including a skew cell age distribution and the hypothesis of heat-induced erythrocyte destruction as a cell age dependent function, will explain a decreasing relative Hb loss with increasing prefebrile anaemia. The delineation of the cell age profile in relation to anaemia in Hodgkin s disease as in column *B* is in accordance with general concepts of erythrokinetics in malignancy (10) especially in Hodgkin s disease (3).

In accordance with the assumption of a self limited process, the effect of fever is not very marked showing a mean Hb loss of 14% and a maximum loss of 33%. In this context it is interesting that the anaemia seen in a number of diseases with different aetiology and with fever as a central clinical feature, such as chronic infection collagen disease, and malignancy is never very pronounced. The mechanism of this type of anaemia, for which the term anaemia of chronic disorders is generally used has never been clarified (1 ?)

The result of fever has little practical implication

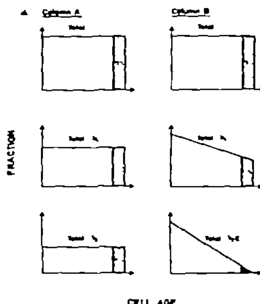


Fig 6 Model constructed in an attempt to explain the influence of the prefebrile Hb level on fever-induced erythrocyte destruction in Hodgkin disease.

since it is rarely necessary to use blood transfusions to correct the anaemia seen in relation to fever. This moreover might be of limited value due to the transfusion of more erythrocytes sensitive to the effect of fever. An attempt to maintain a normal Hb level especially with old blood during fever will require disproportionately many transfusions and hence increase the risk of complications.

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THERAPEUTIC EFFECT OF LEO 1031 AN ALKYLATING CORTICOSTEROID ESTER IN LYMPHOPROLIFERATIVE DISORDERS

I *Chronic Lymphocytic Leukaemia*

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Abstract Leo 1031 chlorambucil ester of prednisolone, has been administered orally to 15 patients with chronic lymphocytic leukaemia (CLL) continuously for 1–29 months (mean 12.5) Seven patients were previously untreated and eight had been treated with prednisolone radiotherapy and/or alkylating gents The initial daily dose was generally 8–16 mg and the maintenance dose was 6–8 mg. Allopurinol was given concurrently I 14 of 15 patients reduction of the leucocyt count was observed and a reduction, in most instances of lymphadenopathy or splenomegaly or both. In seven patients the Hb concentration was improved Significant toxic effects on bone marrow function have been observed in one patient. Two patients developed urticaria. Our study suggests that the drug is effective in the treatment of CLL.

The use of a chemical combination of hormonal and alkylating agents seems logical in the treatment of several neoplastic disorders These two types of drugs act differently within a given cell and may also affect different types of cells within a tumour mass (1)

With this as a starting point, a series of corti coid-alkylating agents was prepared by the Research Laboratories AB Leo Helsingborg, Sweden The synthesis of substances of this type has been reported by Könyves and Kristensson (8) and the relation between chemical structure and cytostatic activity by Könyves et al (7). Among these compounds the pregna-1 4-diene 3,20-dione 11 17,21-trihydroxy ,21-4-p-(bis-(2-chloroethyl)-amino)phenylbutyrate (Leo 1031 EORTC 1502 NSC 134087 CB 400-037 p INN prednimustine) was selected for further studies The structural formula is presented in Fig 1 Similar compounds have been described (2 9 12) but no corticosteroid-cytostatic compound of the same type as Leo 1031 has been reported

Because this new type of ester is lipophilic and differs markedly in physiochemical properties from the parent alkylating acid it might be expected that the drug remains unaltered for a sufficient length of time to reach tumour sites that are inaccessible to the lipophobic free acid (5) The main reason for employing a corticosteroid as a carrier is to obtain a selective distribution, by localizing the alkylating agent in higher concentration within the malignant cells and in this way reduce the systemic toxicity Such specific high-affinity binding of corti costeroids has been demonstrated .g. in lymphoma and CLL lymphocytes (6)

The effect of Leo 1031 on nine animal tumours has been described. The therapeutic indices (T I) were higher than those of chlorambucil in the major ity of cases because of the lower toxicity of Leo 1031 The T I of Leo 1031 in solid Wa 256 carcinoma is about twice that of the equivalent mix ture of prednisolone and chlorambucil (10) Evenaar et al (3) found that Leo 1031 caused a olume reduction of transplantable osteosarcoma of the same magnitude as observed with nitrosoureas

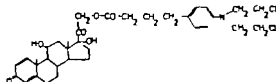


Fig 1 Structural formula of Leo 1031

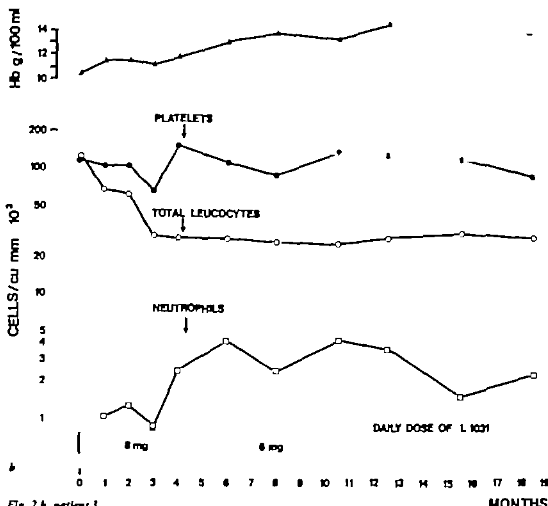


Fig. 2 b. patient 3

hough in one patient (no. 8) the Hb later fell to initial level. Transient thrombocytopenia was ... in patient 10 concomitantly with urticaria after 8 weeks' treatment (see Side-effects). Granulocytopenia was not observed in any patient. Two patients (nos. 11 and 12) had previously received localized radiation therapy. Patient 11 has had marked symptoms of chronic bronchitis and emphysema for about 30 years. Treatment with Leo 1031 caused a reduction of leucocytosis, but the mild anaemia did not improve. Thrombocytopenia or granulocytopenia was not induced. Patient 12 had dermatomyositis complicating her haematologic disease. She had only a moderate leucocytosis which was not significantly reduced during treatment with Leo 1031. There was an increase in Hb concentration and no effects on the thrombocyte or granulocyte counts were observed. The symptoms elicited by dermatomyositis did not improve during the follow-up period.

Three patients (nos. 13–15) had been treated previously with other alkylating agents. Patient 13 was administered chlorambucil 6 mg daily for 2 weeks but the drug was withdrawn owing to thrombocytopenia. This patient was also given prednisolone without improvement of the haematologic data. During treatment with Leo 1031 for 6 months a reduction of the leucocyte count was noted. There was however no improvement in her anaemia, and the thrombocyte and granulocyte counts became extremely low. This patient had a severe splenomegaly and splenectomy was performed. After splenectomy the Hb concentration rose and the thrombocyte and granulocyte counts were substantially improved during continuous administration of Leo 1031. This may indicate that the initial deterioration as reflected by the haematologic data, during treatment with the drug was probably not due to a toxic effect. Patient 14 had undergone treatment with cyclophosphamide

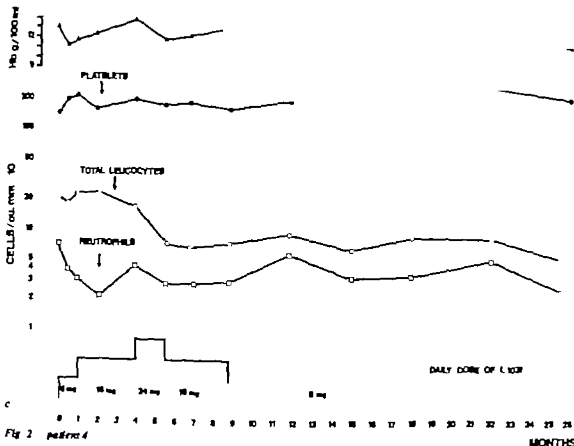


Fig 2 patient 4

for 40 months and had an increasing leucocytosis a falling Hb concentration and decreasing thrombocyte counts. During treatment with Leo 1031 there was a decreased leucocytosis. Thrombocytopenia persisted and the Hb concentration was successively reduced. The patient eventually succumbed with severe anaemia and septicaemia. Patient 15—who previously had been prescribed chlorambucil, 8 mg daily for a short period without effect on her anaemia—did not improve during treatment with Leo 1031 although the leucocyte count was reduced

Side-effects

In two patients (nos 7 and 10) urticaria and fever developed after 3 and 8 weeks treatment, respectively In patient 7 anaemia was aggravated during the reaction and transfusions were given. Granulocytopenia, 800/µl was also noted in this patient, but it could not be determined with any certainty whether the number of granulocytes was reduced from the pretreatment value Thrombocytopenia was found prior to treatment and was not significantly aggravated during the period with fever and urticaria. In patient 10 a transient thrombocytopenia, 75 000/µl was associated with the skin reaction. It must be emphasized that both patients were receiving allopurinol concurrently with Leo 1031 and it is therefore uncertain whether the side-effects were due to Leo 1031 or allopurinol The drugs were withdrawn and the further course has been uncomplicated in both patients The Hb concentration in patient 7 has risen spontaneously to 11 4 g/100 ml during a follow-up period of 12 months after the skin reaction

Patient 9 experienced severe pain in the left shoulder joint shortly after the treatment started In spite of the concurrent treatment with allopurinol serum uric acid was elevated and a clinical diagnosis of gout was made. Following treatment with oxyphenbutazone for 3 days the pain was relieved and has not reoccurred during continuous treatment with Leo 1031

Gastrointestinal symptoms have not been re

ported by any patient No clinical signs of adverse steroid effects e g oedema or increased tendency to bruising, have been observed

DISCUSSION

There seems to be little evidence that treatment of CLL significantly prolongs survival (4) and the main purpose of the therapy is to control troublesome symptoms caused by the disease Therefore a therapeutic regimen without serious side-effects is desirable Animal experiments with Leo 1031 indicated a low toxicity of the substance combined with a high therapeutic index in various experimental tumours (3 10) and it was therefore considered justified to test the effect of the drug in CLL.

Leo 1031 was found to cause a significant reduction of the number of circulating lymphocytes as well as a reduction of enlarged lymph nodes and splenomegaly Signs of bone marrow toxicity have been essentially lacking and in several patients an improvement in the anaemia has been observed The drug has probably not induced thrombocytopenia in any patient, although low platelet counts were noted in one patient in connection with an allergic reaction. Granulocytopenia has been observed in a few patients but it is uncertain whether this was due to treatment or to the disease per se Because low granulocyte counts have been found to increase in spite of continuous administration of the drug, it is improbable that Leo 1031 has a ...ificant granulopoiesis-inhibiting effect in the dose given to the CLL patients. This conclusion is supported because the patients exposed to the drug for prolonged periods did not tend to have lower granulocyte counts than those who received the drug for a short period. The low bone marrow toxicity was confirmed in a series of patients with lymphocytic lymphoma who had received higher doses of Leo 1031 (11)

Our preliminary data thus point to a favourable effect of the drug in controlling CLL. Since the disease characteristically runs a slow course our observation period is however still too short to permit evaluation of the long-term effect of treatment with the drug

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THERAPEUTIC EFFECT OF LEO 1031 AN ALKYLATING CORTICOSTEROID ESTER IN LYMPHOPROLIFERATIVE DISORDERS

II Lymphocytic Lymphoma

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Abstract Leo 1031 chlorambucil ester of prednisolone, has been administered to 20 patients with generalized lymphocytic lymphoma (LL) of various histologic types. The average daily dose was 40 mg orally: the treatment was given continuously for 1-17 months (mean 7). Complete remission was obtained in five patients and partial remission in ten. The best results were obtained in patients with nodular type of LL. Significant leucopenia was induced in two patients no thrombocytopenia has occurred in any patient. I two patients cushingoid habitus developed after 8 and 4 months respectively Leo 1031 may be of value as the single drug in the treatment of some types of LL. A combination therapy with Leo 1031 and vincristine has been tried in 5 of these patients, in 2 as initial therapy and I 3 later in association with relapse. Further trial to assess the value of Leo 1031 as part of a combination schedule are desirable.

Leo 1031 a chlorambucil ester of prednisolone was synthesized in 1969 at the Research Laboratories AB Leo Helsingborg, Sweden (1., 13) and was made available for clinical trial in 1971 (14) In previously published reports (2, 3) the effect of the compound was evaluated in patients with chronic lymphocytic leukaemia. The present report deals with the effect of Leo 1031 in lymphocytic lymphoma (LL)

Previous investigations indicate that poor results can be expected from the treatment of LL ("lymphosarcoma") when employing single drug chemotherapy Thus complete remission rates of 10-20% have been reported with chlorambucil cyclophosphamide or Vinca alkaloids alone (4 8 11). With combination therapy more encouraging results have been obtained, with a complete remission rate of about 90% (9 15 16) These programmes however often require hospitalization for certain periods. Since Leo 1031 contains two of the components usually employed in these programmes i e. alkylating agent and prednisolone, we considered it justifiable to investigate whether the effect of Leo 1031 in LL might be comparable to results reported with more elaborate programmes.

MATERIAL AND METHODS

P tients

Treatment with Leo 1031 was started in 23 patients with LL. Three patients were excluded from this study for various reasons. one patient (no. 4) turned to homeopathy after 2 weeks treatment with Leo 1031 one (no. 5) died 4 days after starting treatment, and in one (no. 18) the cytologic diagnosis was changed to histiocytic lymphoma Of the 20 patients thus included in the study 8 were women and 12 men, with an age range of 29-80 years (mean 60)

Indication for treatment was in all patients generalized disease, e. stages III and IV according to the Ann Arbor classification (5). The staging procedure included in all patients chest X-ray scintigraphy of the liver and spleen bone marrow biopsy and fine-needle aspiration biopsy of the liver spleen, palpable lymph nodes and other sites suspected of involvement. I addition, lymphography and inferior cavography were performed in most patients, but staging laparotomy was not included in the programme. A a result of the staging procedure 8 patients were considered to have stage III disease and 12 stage IV In the latter group involvement of the bone marrow was confirmed in 8 cases, of the gastrointestinal tract in 4 of the skin and subcutaneous tissue in 4 of the skeleton in one

patient, and two patients had malignant pleural and peritoneal effusions respectively

All diagnoses were confirmed through examination of a gross biopsy specimen and the lesions were classified according to Rappaport et al. (17). Of the nodular pattern, two patients had a well differentiated type of LL (NLWD) two a poorly differentiated type (NLPD) and two a lymphoma of mixed lymphocytic–histiocytic type (NM) Of the diffuse pattern, two patients had a well dif ferentiated LL (DLWD) 11 poorly differentiated lymphocytic lymphoma (DLPD) and one a mixed lymphocytic–histiocytic type of lymphoma (DM). This distribution of the different types of lymphoma deviates from that usually reported (10) and may reflect non-intentional selection of patients.

Leo 1031 was given as the first therapeutic measure to 11 patients. Nine patients had been treated previously viz. five with irradiation of localized disease, one with alkylating agents and three with both these methods.

Blood counts were performed in all patients biweekly or monthly during the treatment. Leucopenia was defined as a WBC count below 000/µl and thrombocytopenia as a platelet count below 100 000/µl

Administration of the drug

The drug was available in tablets containing 8 or 20 mg. At the beginning of the study the dose was kept rather low 4–24 mg daily (patients 1–5). The dose level for the remainder have aried between 32 and 80 mg daily 40 mg being the most common dosage. Treatment was given continuously and even in the patients in whom a complete remission was obtained, maintenance therapy was given with the same dosage

Table I *Result of treatment with Leo 1031*

Type of LL	No. of pats.	Remission Complete	Partial	None
Nodular				
NLWD		1	1	
NLPD	2			
NM			1	1
Diffuse				
DLWD	2		2	
DLPD	11	2	6	3
DM	1			1
Total	20	5	10	5

Five patients were given vincristine (Oncovin®) concorrently with Leo 1031 In two initially and in three later in the course of treatment in order t achieve reinduction of remission. In all except one a cyclic schedule was tried Leo 1031 was given for 2 weeks i a dose of 40 mg/m daily and vincristine was given in dose of 1.2 mg/m i on days 1 8 and blood counts permitting, on day 15 After a 2 week pause the next series was started

RESULTS

The patients have been followed for 1–17 months (mean 7) as indicated in Fig. 1 The degree of re sponse has been evaluated according to the

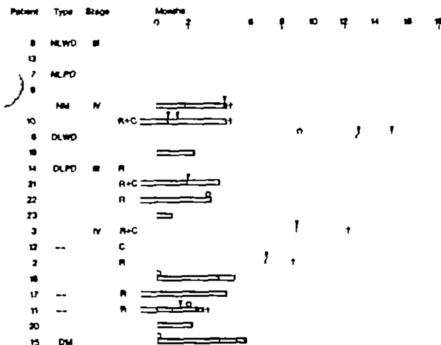


Fig 1 Follow-up of 20 patients with lymphocytic lymphoma treated with Leo 1031 The numbering of the patient indicates the chronological order of start of treatment. *R* = previous radiotherapy *C* = previous chemotherapy

E.O R.T C protocol (6) and the results are summarized in Table I

Complete remission was experienced by five patients (nos. 7 8 9 12 and 14) and all of them are still in remission after 7–17 months (mean 1) One patient had an NLWD type of lymphoma, two had an NLPD type and two a DLPD type. Four patients were in stage III and one in the DLPD group was in stage IV

Partial remission (>50% reduction of tumour mass for >1 month) was recorded in ten patients (nos 1 2,3 6 13 16 17 19 20 and 23) One had an NLWD one an NM two a DLWD and six patients



Fig 2. Scintigrams of liver and spleen (with 99Tc-sulphide colloid) in patient no. 13 with NLWD lymphoma. Cytologically confirmed involvement of the spleen. (*a*) At start of treatment, (*b*) after 3.5 month of treatment with Leo 1031 at dose level of 40 mg daily

a DLPD type of lymphoma. Two patients were n stage III and eight in stage IV Four of the patients relapsed after 4–9 months (mean 7) whereas the other six are still in remission, 1–7 months after the start of therapy Five of them have returned to a normal performance status. An example of the effect on spelnomegaly owing to lymphomatous infiltration is presented in Fig. 2.

One patient (no 6) displayed signs of progression after 9 months treatment and was then started on the Leo 1031+vincristine combination schedule described. This resulted in a new remission, which was regarded as complete and is still continuing 8+ months after induction. Maintenance therapy however had to be discontinued because of a second malignancy a well differentiated squamous cell carcinoma at the base of the tongue necessitating radiation treatment. During this period no progression of the lymphoma was observed. This group also includes one patient (no 16) with a pleural effusion due to a well differentiated mucinous adenocarcinoma of unknown origin. Progress of this tumour was noted during treatment with the combination therapy

Non-total failure (<50% reduction of tumour mass or partial remission for <1 month) was observed in three patients (nos. 15 1 and 22) Two had a DLPD and one a DM type of lymphoma. Two patients were in stage III and one in stage IV In one patient (no 15) the disease is stationary 5+ months after induction with Leo 1031+vincristine but the other two relapsed after 2 and 3 months respectively

Total f llure was noted in two patients (nos 10 and 11) with NM and DLPD lymphoma, respectively In T ble I the non-total failure and total failure groups are pooled in a no remission category

During the follow-up, five patients died 3–12 months (mean 6) after starting treatment with Leo 1031 (F g. 1) Three of them (nos 1 2 and 3) were treated initially in the study with a low dose.

Side-effects

Except i two cases described below significant leucopenia has not been induced, not even after prolonged treatment. Thrombocytopenia has not been induced in any patient. Bone marrow biopsy in one patient (no 8) displayed a normal cellular picture after more than 15 months of continuous treatment with 40 mg Leo 1031 daily

In one patient (no 3) a sudden drop in the WBC

count was noted after 9 months therapy with a daily dose of 24 mg. This occurred however at the same time as local irradiation was administered to a small skin manifestation on the left upper arm The WBC count was 800/μl as a minimum but rose within 1 week to a normal level

In another patient (no 12) a sudden drop in WBC to 200/μl was noted after 3.5 months treatment with 40 mg Leo 1031 daily The leucopenia was observed concurrently with a transient lowering of the BP and a reversible state of mental confusion The WBC count normalized after 2 weeks and treatment with Leo 1031 has been continued for 5+ months

In two patients (nos 15 and 16) for whom the cyclic schedule with Leo 1031 and vincristine was adopted as the first treatment the lowest WBC count was 1000/μl The neurotoxic effects of vincristine were however already very prominent after the first two series and the combination was abandoned Treatment was continued with Leo 1031 as a single drug therapy

In two patients (nos 7 and 13) receiving 40–64 mg Leo 1031 daily a cushingoid habitus developed after 8 and 4 months respectively In one of them a tendency to oedema of the hands and development of cutaneous fibroma were also observed Minor gastrointestinal disturbances such as a periodic diarrhoeal tendency have been observed in some of the patients

DISCUSSION

the present study 15 of the 20 patients with eneralized LL responded to treatment with Leo 1031 The remission was complete in five patients and partial in 10 As could be expected from previous experience (1 11) the best results were obtained in patients with a nodular type of LL whereas the response in patients with a diffuse type was less satisfactory The average required dose was 40 mg daily

The absence of serious side-effects permitted an ambulatory therapy in practically all the patients This indicates that the drug is suitable in patients for whom long-term treatment with alkylating agents may be indicated Furthermore in the event of relapse during such treatment a more aggressive regimen can be started in patients with an intact bone marrow This relative lack of toxicity is an accordance with published experimental observations (7)

The present preliminary results indicate a favourable effect of Leo 1031 in LL, and further trials are therefore justified. For certain types of LL a continuous therapy with Leo 1031 as a single agent may prove adequate but for others a combination therapy is probably more reliable (18) We have tried Leo 1031 as a component in a combination schedule but our experience from this mode of treatment is too limited yet to warrant any conclusions. Further attempts should therefore be made to assess the value of Leo 1031 as part of a combination chemotherapy programme

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Acta med. scand Vol 197 pp 329–335 1975

RENIN SECRETING RENAL TUMOUR WITH SEVERE HYPERTENSION

Case Report with Tumour Renin Analysis Histopathological and Ultrastructural Studies

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E. K Brodwall and A Flatmark

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Abstract A 25-year-old man presented with severe hypertension associated with hypokalaemia, elevated plasma renin level and secondary hyperaldosteronism. Malignant phase hypertension and renal artery stenosis were ruled out, and a preoperative diagnosis of renin-secreting renal tumour was made on the basis of higher concentrations of renin in the left than in the right renal venous plasma in spite of normal findings on selective renal arteriography By removal of the affected kidney the tumour was found and it had very high content of renin. Following the operatio the plasma renin level serum aldosterone concentration and BP became normal. W present a histopathological description and an ultrastructural study of the tumour

Hypertension due to renin-secreting renal tumour was first described by Robertson et al. (11) in 1967 Three months later another case was described by Kihara et al (10) and five cases have been reported later (3 4 6, 8 13) In all cases the tumour has histologically been described as hemiangiopericytoma with cells resembling the juxtaglomerular cells After surgical removal of the tumours remission of severe hypertension has occurred in all cases.

In the present report we describe a patient n whom the diagnosis was made preoperatively Much higher renin concentrations were found in the tumour than reported at the time of investigation. Furthermore, the BP and the plasma renin activity (PRA) were closely followed during the postoperative period demonstrating a correlation between these two parameters The tumour has also been characterized by light and electron microscopy

METHODS

Plasma renin activity

PRA wa measured according to Haber et al (9) with some modifications as described by Cherva et al. (5) Blood was draw in precooled EDTA vials. The plasma was incubated at 37°C (blank at 4°C) for 3 hours and PRA was calculated from the angiotensin I formation. The angiotensin I formed was measured by radioimmunoassay with commercial kit (Angiotensin I Immunotope Kit from E. R. Squibb). Conversion of angiotensin I to angiotensin II was inhibited by the addition of EDTA dimercaprol and hydroxyquinoline. The upper normal limit for PRA in non-diseased person in supine position is by this method pproximately 1.5 ng/ml/h (7).

Renin activity in tissue

The tissue was frozen immediately after excision After thawing it was cut into small pieces and homogenized in Potter-Elvehjem homogenizer in 40 ml of 0 9% sodium chloride/g wet weight of tissue. After 30 min on ice with gentle movements of the pestle 6 times the homogenate was centrifuged $800 \times g$ for 5 min. The pellet was discarded and after appropriate dilution with 0 9% sodium chloride the supernatant was assayed for renin activity Supernatant from 1.25 mg of normal kidney cortex or from $1.25 \quad 10^{-6}$ to 1.25×10^{-4} mg of the tumour was used in the assay with plasma as substrate. Blank were run with the same amounts of boiled supernatants (10 min in boiling water) Renin standard was purchased from Medical Research Centre Holly Hill London One unit of renin activity gave in our hands the formation of approximately 19 000 ng angiotensin I/h

Histological preparation

The material was fixated in buffered formol saline and Bouin fluid, processed embedded in paraffin and sectioned. The following stains were used haematoxylin-erophloxine-saffron, periodic acid Schiff reagent and Wilson stain for demonstration of the granules in the juxtaglomerular complex.

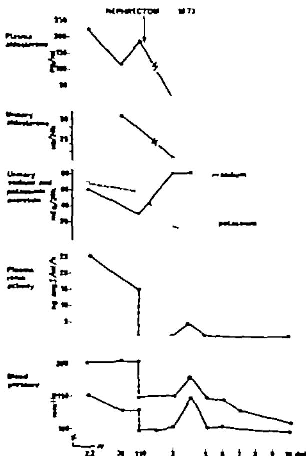


Fig 1 Relationship betwee plasma aldosterone concentration urinary aldosterone sodium, and potassium excretion PRA and BP before and after removal of the renin-producing tumour

ctural studies

specimen was fixed in ..5% glutaraldehyde in 0 1 M osphate buffer (pH 7 4) for 4 hours and postfixed in 1% osmium tetroxide in Tyrode solution (pH 7 4) for hours

Dehydration was carried out in graded ethanol and th material wa embedded in Epon 812 Semithin and ultrathin sections were cut with an LKB ltrotome ultramicrotome The semithin sections were stained with Toluidine-blue and the ultrathin sections w th uranyl acetate and lead citrate The section were examined in a Jeol 100 B electron microscope

CASE REPORT

The patient is a ..5-year-old man previously in good health the BP being normal in 1969 During the last two years he had suffered from episodes of headache and remarkable thirst. In Dec. 197? he had two episodes with transient speech difficulties and paresthesia in the right hand, and an elevated BP (200/150 mmHg).

He was admitted to hospital in Jan 1973 and apart from hypertension 180–220 mmHg systolic and 140–170 mmHg diastolic findings of the physical examination were normal ECG showed left entricular hypertrophy and the chest X-ray was normal without heart enlargement. There were retinal exudates and hemorrhages with narrowing of the arterioles but not papilloedema. Serum sodium as 139 potassium ..9 chloride 96 and tandard bicarbonate 3? mEq/l. Creatinine clearance was 80 ml/min and except for slight proteinuria the urine was normal. Rapid sequence L pyelogram and renal arteriogram were both normal Isotope renogram showed symmetrical, delayed ex cretion. Both PRA and serum aldosterone were elevated (Fig 1)

In spite of heavy medication with hydralazine a-methyldopa propranolol, trichlormethiazide and for a period spironolactone the BP was not satisfactorily regulated with diastolic values of about 110–130 mmHg while on a diet containing approximately 10 mEq sodium/day

Bilateral renal ein catheterization was performed and very high PRA levels were found on the left side Renin activity i enous blood from the right kidney was, supine 23.5 upright 4.5 and from the left kidney supine 65.3 upright 51 9 (g ang I/ml/h) Based on the abnormal production of renin in the left kidney the diagnosis of tumour was made and operation was advised in pite of normal findings on a new selective renal angiography with tomography

On Oct 1 1973 the left kidney was removed and the tumour was found Within half an hour the BP fell to normal levels (Fig. 2) and remained normal on the first postoperative days. From the 3rd to the 10th postoperative day he had a transient rise BP t maximally 185/145 mmHg on the 3rd postoperative day (Fig 1) Antihypertensive therapy wa necessary for one week and during this period the BP varied during the day and rise in BP was accompanied with flushing and tachycardia Later the BP has been normal (110–130/75–90 mmHg) without medication for one year

The PRA fell rapidly after removal of the left kidney (Fig. ?) with a half-life of renin in plasma of approx mately 100 mm After 8 hours PRA was barely measurable PRA followed the BP in the postoperative phase with rise up to 4.5 ng/ml/h on the 3rd postoperative day PRA remained increased for one week and became normal simultaneously with the BP (Fig. 1) Postoperatively the serum aldosterone concentration and aldosterone excretion became normal and accordingly the urinary excretion of sodium and potassium and serum potassium concentration were normalized (Fig. 1)

RESULTS

Renin studies

The PRA was between 15 and 25 ng/ml/h on several occasions over a period of seven months (Fig 1) At renal vein catheterization very high renin activity was found on the left side Renin activity in the right renal vein was similar to PRA as could be expected.

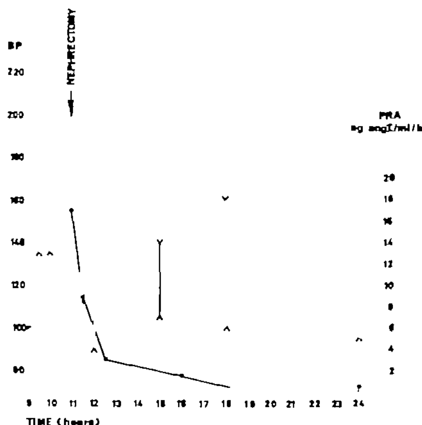


Fig 2 Relationship between PRA and BP on the day of operation

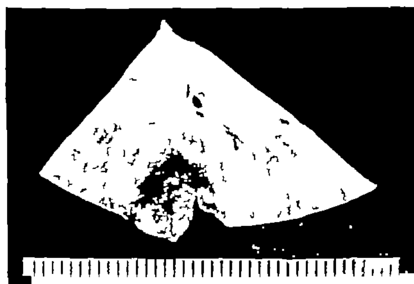


Fig 3
with

BOOK REVIEW

Trac elements in relation to cardiovascular diseases Edited by R. Masironi. 45 pp Sv fr 7.00. WHO offset Publication No. 5 Switzerland 1974

This booklet i based on the reports of three meetings (Geneva, Feb 1971 Vienna, Feb 1973 and Geneva, April 1973) of investigators on trace elements in relation to cardiovascular disease held jointly by the International Atomic Energy Agency (IAEA) and the WHO

The subject has attracted increasing interest in recent years. WHO together with IAEA are coordinating re search work on an international basis and the following six projects have been started 1) Trace elements in human tissues in relation to ischaemic heart disease 2) Cadmium and zinc in relation to hypertension. 3) Living population studies. 4) Trace elements in nutrition. 5) Relationships between cardiovascular death rates and chemical characteristics of local water supplies. 6) Geochemically oriented studies in relation to cardiovascular diseases.

Thi booklet presents the background of these studies and the applied methodology of trace element analysis. The results to date are also presented and discussed but are still too few to be conclusive. Finally the booklet presents recommended approaches for internationally coordinated investigations on trace elements in relation to cardiovascular diseases The booklet thus provides easily accessible information in thi field of research and it is recommended not only t specialists in trace elements but also to colleagues interested in the pathophysiology of cardiovascular diseases

P O Wester Stockholm, S eden

Acta med. scand Vol 197 pp 337–338 1975

EDITORIALS

HIGH BLOOD PRESSURE AND PREVENTION OF STROKES

The demonstrations by the Veterans Administration Cooperative Study (6 7 8) that lowering of the blood pressure in hypertensive men caused a significant decrease in hypertension-related morbid events have led to a world-wide enthusiasm for treating patients with raised arterial pressure This has been further stimulated by the introduction of several new PB-lowering drugs that seem to promise easier treatment with fewer side-effects. This is true even though much more experience is needed before the exact position of these drugs within the therapeutic arsenal is firmly established

The background to the present optimism regarding treatment of high BP is well outlined in the accompanying Supplement to this issue of Acta med. scand. Within the general frame of prevention of some frequent disorders in this case vascular complications of arterial hypertension Ed Freis presents a summary of the VA studies He also demonstrates that lowering of the BP in the genetically hypertensive rat during a limited part of its lifetime is followed by a lower BP after treatment is stopped. For the rest of their lives the arterial pressure of treated rats was lower compared with their litter mates

It is also shown that this relative decrease in BP is accompanied by less vascular changes of the arterial tree compared with those in the control group Similar data have been published by other groups.

In this part of the symposium Ralph Reader from Australia briefly describes the present efforts all over the world to find early cases of raised BP in order to institute early treatment with the hope of preventing late vascular complications, especially cerebrovascular catastrophes. The difficulties inherent in this approach are well illustrated and the preliminary character of the experience to date is well outlined This is also evident from the contribution to the symposium from Lars Wilhelmsen who describes an ongoing Swedish study running in Göteborg. Its aim is to screen part of the middle aged male population for risk factors deemed to be of importance for the development of arterial disease and its complications. Persons identified as having risk factors of importance are offered treatment and it is planned to compare the outcome for the screened group with control population of the same age This study is in its 4th year and the preliminary results demonstrate in particular the prevalence of high BP in a Swedish middle aged male population, the difficulty of deciding the point at which BP should be defined as raised and the varying interest in health problems displayed by middle-aged males

The latter problem was also emphasized by other participants in the symposium notably J N Morris from London who thought that a new strategy for health had to be defined, with better and more realistic health education starting early in school

Although the results of the VA studies of treatment of high BP have been criticized the general feeling all around the world today seems to be that the time has come for a concentrated effort aiming at early treatment of high BP in large parts of the population. Whether this should be done through screening of the total community—at least above a certain age—or through other community-based programs is still an open question The discussion provided in the accompanying Supplement to this issue of Acta med scand does not answer this or several other difficult questions. But it should serve as impetus to more official bodies responsible for health policy to consider the question.

The many studies conducted in Europe and elsewhere in conjunction with the European Office of WHO after its initiative in creating a community

the beginning of the 1950ies made it possible to produce larger quantities of antigen of different viruses. Since the major problem concerning infectious diseases in those days was poliomyelitis the interest was first focused on the possibilities to combat this disease by vaccination. Remarkable achievements have been made by the use of first the formaldehyde inactivated vaccine and later in most countries the live virus vaccine. In countries with a highly developed health care system the potential use of only inactivated vaccine to provide a medically highly safe means of immunization has been exemplified From the global point of view poliovirus infections still present a major problem It remains to overcome the complications posed by intercurrent enteric infections which in developing countries extensively restrict the frequency of successful immunization with live virus vaccine

Subsequent to vaccines against poliomyelitis means for active immunization against different childhood diseases were developed Measles was first on the list and experiences available today have clearly demonstrated that the available live virus vaccines are safe and provide a long-lasting immunity The vaccine is extensively used in various industrialized parts of the world but there are urgent needs for the introduction of vaccine in developing countries in which measles still remains one of the most common diseases of childhood

Rubella virus was not isolated until 1962 but within a few years attenuated vaccine strains had been developed Vaccination against rubella has a special haracter since the problem concerns not primarily protect the vaccinated individual but rather the veloping fetus of possible future pregnancies vailable accines appear harmless but there are still some questions concerning the duration of immunity and the risk for fetal damage upon accidental vaccination during pregnancy Varying policies of vaccinations have been established in different countries There are two major differences The first concerns the question whether a herd immunity should be developed. The second attempts selective protection of individuals at risk. During recent years a live attenuated mumps virus vaccine has also become available. This vaccine initiates a harmless infection and immunity as far as it has been studied appears satisfactory

Certain negative experiences have been made in attempts to develop inactivated vaccines against different viruses of the respiratory tract In some cases vaccination with what might have been antigenically defective products have established a worser situation than the absence of vaccination.

Further developments in this field are expected to take somewhat different courses It should be emphasized that respiratory infections are of major importance both in developed and in developing countries

There are many reasons to be optimistic concerning the future improvement of available and the development of new vaccines The new vaccines will aim at giving protection against important diseases e g. those caused by hepatitis virus various herpes viruses and tumour viruses. Eventually we may be confronted with a relatively overloaded vaccination calender and there will be an increasing need to arrange for simultaneous immunization against more than one disease. Still the number of future problems in providing a broad range prevention of virus diseases should not be underestimated In each case individual evaluations will have to be made of the need for introducing a general vaccination and also of the safety of the products which may be developed It is important that a general consensus is reached in the formulation of general principles for the application of a product.

Decisions on introducing preventive measures in the form of general vaccination carries with them considerable responsibilities The consequences with regard to prevalence of disease and to circulation of virus in the community as well as to the chances for and the risk of challenges of the epidemiological situation need to be appreciated

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BOOK REVIEWS

Symposium on Myocardial Metabolism. Amer Heart Assoc monograph no 44 Guest Editor Eugene Braunwald. Originally published in Circulation Research. 215 pp $6.00

This monograph is interesting from many points of view It is the result of a joint meeting between American and Russian cardiologists. The president was Eugene Braunwald Professor of Internal Medicine at Harvard The leading Russian investigator was E I Chazov Deputy Minister of Health Moscow The exchange of ideas was lively and the discussions are well worth printing. The subject is rather new and very sophisticated medical science that may be called molecular cardiology and the morphology is all of electron microscopic dimensions. The reviewer is reminded of question he received from one of the professors and department heads of medicine in one of the leading American universities some 15 years ago. Who should we have as the next head of cardiology perhaps an enzymologist?

This monograph opens up very promising field in circulation research and demonstrates the importance of biochemical thinking also in connection with the most mechanical of all the organs, the heart pump Disturbed homeostasis in the enzyme-spending systems must be fundamental problem in attempting to repair the machinery

Many of the chapters make excellent reading and it is difficult to choose some special ones. Braunwald discusses problem of equal importance to the general practitioner and to the cardiac physiopathologist, namely the factors that may induce a reduction of the infarct size after coronary occlusion It is probable that clinical applications of the findings in this group may one day have decisive influence on the fate of the patient with recent infarct.

Several papers deal with the fundamental changes in protein synthesis that allow cardiac hypertrophy to take place One group discusses the RNA changes during this process, another the metabolism of myosin both anabolic and catabolic leading t an increase in content during hypertrophy Morgan and his group treat similar problem with the aid of the perfused rat heart. The protein turnover has been studied in detail and the conclusion is that cardiac muscle cells appear t have a lif that may be as long as the organ itself On the other hand molecules in this cell have rapid turnover of 5–1 d ys. It is an interesting fact that the myocardial cells represent only 25% of the total cell number in the myocardium They occupy about 75% of the total olume. The role of creatine as stimulator of amino acid incorporation into the myosin molecule is discussed. If the resistance for the left ventricle is increased it seems as if the half-life of the myosin molecules decreased to only 3 days, whereas the same value for myosin in skeletal muscle under normal conditions is 28–30 days

Excellent electron microscopic pictures illustrate the relation of form and function on the subcellular some times even on the molecular level. It is impossible to quote more papers from this fascinating volume that allows vista that stretches far into the future of medicine in general and especially into the cardiology of new generations of investigators and practising physicians.

Jan G Waldenström

Brain Dysfunction in Metabolic Disorders Edited by F Plum. 324 pp US$25.00 Elsevier/Excerpta Medica North-Holland, Amsterdam and New York 1974

This volume is another example of the recent interest in metabolic processes in an organ that was almost terra incognita in this respect only some decades ago Brain dysfunction in metabolic disorders is rather modest title for book that treats so many problems of important metabolic dysfunction of the brain The reason, why study of disturbed metabolic homeostasis has been the basis for nearly all great improvements in medical therapy is evident. Biochemical imbalance may often be adjusted either by addition or removal of identifiable substances. One of the reasons why so many serious disturbances of brain function have been regarded as intractable has been the passive acceptance of disturbed circulation as the most probable basis with the consecutive diagnosi cerebral sclerosis

This book strikes much more optimistic note discussing neurologic changes in liver disease, the importance of ions, water and toxins, different metabolic disorders influencing brain function and finally an analysis of lead intoxication

The acceptance of hepatic coma as metabolic intoxication has lead to some remarkable improvements in therapy These are discussed but it is also stressed that there are still many enigmas. One of them is analysed by Cavanagh who is member of the British Medical Research Council group in applied neurobiology He studies the connection between the role of the astrocyte of glial cell and the clinical picture seen in patients with chronic severe liver disease There are obviously characteristic morphologic changes in this cell that are connected with

liver insufficiency It is tempting to assume that these changes are related to cerebral dysfunction How this fits into the picture of control of ammonium ions is still enigmatic but many hepatic problems now have both a biochemical and a morphological substrate

Another chapter treats the disturbed amino acid pattern in severe hepatic failure and tries to connect this disturbance with decreased synthesis of norepinephrine in the brain It is possible that changes in neurotransmitters is the basis of some cerebral symptoms in hepatic encephalopathy

Several chapters treat the problem of brain dysfunction when the circulation does not offer sufficient basic metabolites such as oxygen and glucose. Siesjö and his collaborators have an excellent chapter on ischemia and hypoxia. It is interesting to note that both these monographs treating the metabolism of heart and brain make such a clear distinction between ischemia and hypoxia. Siesjö stresses the point that the energy stores in the brain are relatively low and the tissue is therefore dependent on continuous supply of blood. It is difficult to study pure hypoxia and its effect on the brain as ischemia is usually also present. Further study of purely anoxic cell damage requires carefully controlled studies.

I will only discuss a few more of th many interesting chapters Fishman treats the complicated problem of ion pumps when he discusses the effects of hyperosmolality and hypoosmolality on the brain. This is a problem that has a very great clinical importance and we have become increasingly aware of hyponatremic coma and the ways of correcting the basic effects It is clear that brain cell behave as modified osmometers. These problems are further treated in the following chapter about uremia with water retention and seizures, the tetany of hypocalcemia and the stupor of hyponatremia. Two chapters are devoted to a genetic disorder causing severe neuroencephalopathy namely the three different acute porphyrias. Recent American investigations have shown that these clinical pictures are caused by a metabolic block in the transformation of porphobilinogen into uroporphyrinogen. When external factors such as drugs or estrogens induce synthesis of the rate limiting enzyme ALA synthetase a number of metabolites increase in blood and tissues. Their role in the development of neuroencephalopathy is still not defined but fundamental knowledge has been obtained Also in this disease disturbances of osmolality may have severe repercussions on brain function. It seems to me that these two chapters illustrate the interaction of genes, enzymes, metabolites and drugs in a perfect way and they will teach fundamental lessons, not only to the neurologist but also to the general internist.

From the point of view of world medicine the problem of malnutrition and its influence on brain development in infants has astronomical consequences. Winick treats this urgent question in a short but excellent chapter

The book may well be said to illustrate the futurology of dynamic basic and clinical neurology and shows the diminished importance of static morphological thinking It may be recommended to clinicians in many fields and the editor and organizer Fred Plum at New York Hospital may well be congratulated on having organized a symposium of such excellent quality

Ja G Waldenström

The mechanism of nergy transduction in biological systems Annals of the New York Academy of Sciences, Vol 227 Edited by David E. Green 680 pp. $40.00. New 1974

) book, like all other New York Academy of Sciences als, s the outcome of a conference at the Academy The book appeared in February 1974 the conference having taken place exactly one year before

It is certainly fitting that Da id E. Green, from the Institute for Enzyme Research in Madison, served as Editor and Conference Chairman. Many of the other authors are likewise well renowned scientists in mitochondrial biochemistry ("mitochondriacs as they sometimes facetiously call themselves). However the scope of the book is by no means limited to the mitochondria and their plant counterparts, the chloroplasts. On the contrary the main thrust is the search for unifying principles of bioenergetics in a wide sense The conceptual framework is provided by a 40-page introductory essay by Green. He emphasizes the fundamental role of protein molecules as macromolecular devices for energy manipulations in catalytic as well as in noncatalytic interactions. Protein molecules can serve this basic function by virtue of their spring-like tertiary structure Thus, the science of bioenergetics becomes the study of the energy-transducing capability of proteins, i.e their ability to convert thermal energy into biologically useful electromechanochemical energy

The other contributors enlarge on various aspects of the living organism s incessant struggle to harness energy from the environment and convert it into useful processes, avoiding excessive heat spillage (thermalization). The relevant changes in protein conformation are described in great detail. Naturally many of the presentations dive deep into physical chemistry thermodynamics and quantum mechanics, thereby often transcending the absorptive powers of most medical biologists.

Considerable space is allotted to considerations of the energy transactions involved in muscular contraction and in excitable membranes. Nachmansohn summarizes his long-held view of the function of acetylcholine This molecule is seen as a signal initiati g the reaction that change ion permeability in all excitable membranes On the other hand Nachmansohn rejects the classical view of cholinergic synaptic transmission: the acetylcholine molecules do not cross the ynaptic cleft, xcitation of the postsynaptic membrane being caused by overbridging ion fluxes. He asserts that Loewi's classical experiments on acetylcholine release cannot be reproduced.

In general the presentations bear no particular reference to the field of medical biochemistry An exception is the

discussion of ionophores by Pressman and de Guzman. Ionophores are biological or artificial molecules that are able to bind small ions, such as metal cations, and carry them through biological membranes. Since biological processes are greatly dependent on transmembrane ion gradients created by ion pumping systems it is not surprising that ionophores have interesting biological effects. This has long been known to be the case with isolated mitochondria. Pressman now describes the properties of novel ionophore with great affinity for divalent cations, but capable of carrying other metals as well and also organic ions like noradrenaline. This compound was found to have profound effects on the performance of muscle cells. The studies to date extend to the intact dog, where a considerable increase in cardiac contractility was elicited, in conjunction with moderate increase in blood pressure and a decrease in total peripheral resistance. Thus this ionophore is seen as possible therapeutic agent, potentially superior to present inotropic agents, i.e., catecholamines and digitalis.

A medical field notable for its absence is the disturbed energetics of hyperthyroidism. The energy loss of this disorder was long felt to be consequence of reduced coupling in mitochondrial oxidative phosphorylation causing diversion of energy from ATP synthesis to waste heat. Rather recently it has been shown that the increased heat production is the consequence of increased ion pumping in all cells. Abnormally steep sodium and potassium gradients are being upheld and the energy involved in this useless work ultimately appears as increased heat production. Certainly pathological biochemistry is no less astounding than the normal.

Jörgen Malmquist, Malmö, Sweden

Acta med. scand. Vol. 197 pp. 345–351 1975

SCINTIGRAPHY WITH ^{131}I 19-IODOCHOLESTEROL IN ADRENAL DISEASE

An Evaluation

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Aker Hospital, Oslo Norway

Abstract Adrenal scintigraphy after i.v. injection of ^{131}I 19-iodocholesterol has been performed in 4 patients with primary aldosteronism, 5 with Cushing's syndrome and 1 patient with phaeochromocytoma. In primary aldosteronism unilateral adrenocortical adenoma was demonstrated in 2 patients, while the method failed in 1 patient to visualize a tumour that was localized by measurements of aldosterone concentrations in the adrenal veins and by adrenal venography. In 1 patient none of the methods demonstrated a tumour. In Cushing's syndrome, adrenal scintigraphy indicated bilateral adrenocortical hyperplasia in 1 patient and visualized the tumour in 2 patients with adrenocortical adenoma. In all patients with Cushing's syndrome due to unilateral adrenocortical tumour the accumulation of radioactivity in the contralateral adrenal was suppressed. However, delayed and slight accumulation of the isotope in the suppressed gland contralateral to an adrenocortical carcinoma was misinterpreted and led to exploration on the wrong side since the tumour did not concentrate radioactivity at all. The method failed in 1 patient to localize the adrenocortical tissue responsible for the relapse of Cushing's syndrome after bilateral adrenalectomy for hyperplasia. In the patient with phaeochromocytoma, no radioactivity was found on the tumour side due to compression of the adrenal cortex by the tumour. It is concluded that adrenal scintigraphy is a safe and valuable method for localization of adrenal tumours and their differentiation from adrenocortical hyperplasia. Some diagnostic pitfalls do however exist, as demonstrated in this series of patients.

The final diagnosis of Cushing's syndrome and of primary aldosteronism is based on hormone assays. In Cushing's syndrome it may also be possible from suppression tests to differentiate between adrenocortical tumour and hyperplasia, whereas this cannot be done in primary aldosteronism (18). It has been shown that bilateral adrenocortical hyperplasia is present in 15–30% of cases with primary aldosteronism (6, 9, 10, 11) and that these patients rarely benefit from surgical treatment (6, 11).

The scintigraphic procedure of Conn et al. (9, 13) with visualization of the adrenal glands after ^{131}I 19-iodocholesterol administration, would in patients with Cushing's syndrome mainly be of importance for tumour localization, while in patients with primary aldosteronism the additional information with regard to the presence of a tumour or bilateral hyperplasia would be significant.

In a preliminary report (21) we presented encouraging results in two patients with primary aldosteronism in whom a unilateral adenoma was visualized by adrenal scintigraphy, thus making elective surgery possible. Further experience with the method has made it clear that the results are not always so unequivocal as in our first two cases. Our total series of 10 cases may be of interest in this connection, pointing to some pitfalls that can complicate the interpretation of adrenal scintigraphy.

METHODS

Plasma cortisol was measured fluorometrically (14), plasma aldosterone (20) and plasma renin activity (19) by radioimmunological methods. The determination of aldosterone secretion rate was performed as described by Aakvaag (1). Urinary 17-hydroxycorticosteroids were determined by the Norymberski procedure (3) with the modifications described by Metcalf (16). Adrenal scintigraphy was performed as described previously (21). Pictures were taken with a gamma camera during a 4–10 days period following i.v. administration of ^{131}I-19-iodocholesterol. The localization of the adrenals in relation to the kidneys was established by gamma camera pictures of the latter during ^{131}I-iodohippuran renography.

Table I *Aldosterone secretion rate (ASR) plasma aldosterone concentration (PA) and plasma renin activity (PRA) in 4 cases with primary aldosteronism*

Case no.	Sex	Age (y)		ASR (μg/24 h) Supine	PA (pg/ml) Supine	PRA (ng ang I/ml/h) Supine	PRA (ng ang I/ml/h) Upright
			Preoperatively				
1	♀	56	Sodium depletion	345	186	0.3	0.9
			Sodium loading	503	205		
			Postoperatively		15		1.6
			Preoperatively				
2	♀	51	Sodium depletion	352	86	0.2	0.2
			Sodium loading	725	108		
			Postoperatively		34		0.8
			Preoperatively				
3	♀	23	Sodium depletion	186	208	0.1	0.1
			Sodium loading	76	133		
			Postoperatively		47		1 1
4	♂	16	Sodium depletion	271	500	0.1	0 1
			Sodium loading	254	171		
Normal values (upright, unrestricted diet)				75–150	25–150		<1.2

RESULTS

Primary aldosteronism (4 cases Table I)

All patients had arterial hypertension, hypokalaemia and low and suppressed plasma renin activity as well as an increased plasma aldosterone concentration and a high aldosterone secretion rate that were not or only incompletely suppressed by sodium chloride (12 g in divided doses on each of 3 days) The criteria for the diagnosis are given in T[illegible]le I Cases 1 and 2 have been reported earlier

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56 years of age. Adrenal vein catheterization was not performed as adrenal scintigraphy showed accumulation of radioactivity almost exclusively in the left adrenal which contained an adenoma, 22 mm in diameter

Case 2

Female 51 years of age A tumour in the right adrenal, 14 mm in diameter was demonstrated by adrenal scintigraphy allowing elective surgical treatment. Previous catheterization of the right adrenal vein had not been successful

Case 3

Female 23 years of age This woman had developed arterial hypertension and hypokalaemia during the year before the examination. Both plasma concentration and aldosterone secretion rate were reduced by sodium loading, but not suppressed to the extent seen normally Following the injection of ^{131}I-cholesterol a symmetrical accumulation of radioactivity appeared in both adrenal regions (Fig. 1). Adrenal phlebography however disclosed an adenoma in the right adrenal, and the plasma concentration of aldosterone was 10 times higher in the right than in the left adrenal vein. The right adrenal, containing an adenoma 20 mm in diameter was subsequently removed Two months postoperatively the serum kalium concentration and BP were normal. The adrenal phlebography and



Fig 1 Case 3 woman with primary aldosteronism and adenoma in the right adrenal. Photoscintigram (posterior view) showing symmetrical accumulation of radioactivity in both adrenal regions. The outline of the kidneys is drawn after ^{131}I-hippuran renography

the adrenalectomy were performed at Telemark Central Hospital, Skien, under supervision of the head of the Medical Department, B Knutsen.

Case 4

Male, 17 years of age. Arterial hypertension and spontaneous hypokalemia were first diagnosed two years before the present study At adrenal scintigraphy radioactivity was symmetrically localized to both adrenals. Scintigraphy repeated after dexamethasone 2 mg/day in divided doses given 2 days before and 2 days after the injection of [131]I-cholesterol showed the same pattern of uptake (Fig. 2). N tumour was demonstrated in the left adrenal by adrenal venography catheterization of the right adrenal vein was unsuccessful. Treatment with spironolactone has recently been started.

Cushing s syndrome (5 cases Table II)

All patients presented clinical signs of Cushing s syndrome The results of the hormone assays are given in Table II

Case 5

Female, 32 years of age. The hormone assays showed the pattern of bilateral adrenocortical hyperplasia. At adrenal scintigraphy the radioactivity accumulated symmetrically in both adrenals. Bilateral adrenalectomy was performed. The weights of the hyperplastic glands were 6 5 and 7.5 g respectively

Case 6

Female, 39 years of age. The biochemical data, although inconclusive, were found to be compatible with the presence of an adrenocortical tumour After injection of [131]I-cholesterol, a considerable accumulation of radioactivity to the right adrenal was found. No radioactivity was detected in the left adrenal, reflecting the suppressed function of this gland. The right adrenal was removed containing an adrenocortical adenoma (weight 25 g) The patient had to be treated with cortisone for several months after the operation.



Fig 2 Case 4 man with primary aldosteronism. Photoscintigram (posterior view) after dexamethasone showing symmetrical accumulation of radioactivity on both adrenal regions. The renals outlined after [131]I-hippuran renography

Case 7

Female, 27 years of age. The biochemical evaluation was inconclusive in the preoperative differentiation between adrenocortical tumour and hyperplasia. Adrenal scintigraphy disclosed accumulation of radioactivity in the left adrenal gland exclusively the right one being suppressed (Fig. 3). The left gland with an adenoma (weight 8 g) was removed. Cortisone had to be given for half year postoperatively

Table II. *Plasma cortisol urinary 17-hydroxycorticosteroids (17-OHCS) and tetrahydro-11-deoxycortisol (THS) in 5 patients with Cushing syndrome*

				Plasma cortisol (μg/100 ml)				Urinary 17-OHCS (mg/24 h)			
				Basal at		8 a.m. after dexamethasone*			After dexamethasone*		Basal urinary THS
Case no	Age (y)	Sex	Adrenocortical pathology	8 a.m.	8 p.m.	I	II	Basal	I	II	(mg/24 h)
5	32	♀	Bilateral hyperplasia	25 0	18.4	15 7	9.8	40.7	19.2	7 1	0 11
6	39	♀	Unilateral adenoma	28 1	23.8	20.4	9.3	44.3	–	16.2	0.13
7	27	♀	Unilateral adenoma	29 7	27.5	27.0	7 4	25 1	28.5	31.6	0 13
8	35	♂	Unilateral carcinoma	71.0	66 5	41 1	39.0	116.0	128.3	124.0	0.69
9	51	♀	Hyperplasia (ectopic)	18 5	20.3	18.2	8.7	20.5	13.2	3 7	0.37

I=2 mg/d. for 2 days, II=8 mg/d. for 2 days.

Table I *Aldosterone secretion rate (ASR) plasma aldosterone concentration (PA) and plasma renin activity (PRA) in 4 cases with primary aldosteronism*

Case no.	Sex	Age (y)		ASR (µg/24 h) Supine	PA (pg/ml) Supine	PRA (ng ang.I/ml/h) Supine	Upright
			Preoperatively				
1	♀	56	Sodium depletion	345	186	0.3	0.9
			Sodium loading	503	205		
			Postoperatively		15		1.6
			Preoperatively				
2	♀	51	Sodium depletion	352	86	0.2	0.2
			Sodium loading	725	108		
			Postoperatively		54		0.8
			Preoperatively				
3	♀	23	Sodium depletion	186	208	0.1	0.1
			Sodium loading	76	133		
			Postoperatively		47		1.1
4	♂	16	Sodium depletion	271	500	0.1	0.1
			Sodium loading	254	171		
Normal values (upright, unrestricted diet)				75–150	25–150		<1.2

RESULTS

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Fig 1 Case 3 woman with primary aldosteronism and adenoma in the right adrenal. Photoscintigram (posterior view) showing symmetrical accumulation of radioactivity in both adrenal regions. The outline of the kidneys is drawn after ^{131}I-hippuran renography



Fig. 5. Case 10, woman with phaeochromocytoma in the left adrenal. Photoscintigram (posterior view) showing concentration of radioactivity in the right adrenal region, but no accumulation on the tumour side.

tients with primary aldosteronism have however been somewhat difficult to reconcile with what is known of the adrenocortical function under normal and pathological conditions. Before discussing the present cases it may therefore be of interest to review some basic facts.

Firstly as evidenced by cortisol determinations in blood and urine the cortisol production in the adrenal cortical tissue is usually not suppressed by an aldosterone-producing tumour unless the tumour is also producing cortisol. *Secondly* since the normal cortisol secretion rate (15–20 mg/24 h) is 20–30 times above the highest aldosterone secretion rate we have measured in a patient with primary aldosteronism it is surprising that the increased uptake of radioactivity in an aldosterone-producing tumour will give rise to a detectable difference in radioactivity between the two adrenals. The observed concentration in the small adenomas of sufficient radioactive cholesterol to be of diagnostic importance is rather difficult to explain.

Irrespective of these considerations empirically the method of adrenal scintigraphy is obviously able at least in some patients to disclose the increased accumulation of radioactivity in such a tumour as was the case in patients 1 and 2. It is however evident from the findings in patient 3 in whom the tumour was demonstrated by adrenal venography only that a symmetrical concentration of radioactivity in both adrenals does not rule out the presence of a unilateral tumour.

Conn et al. (9) have reported on a patient with primary aldosteronism and symmetrical accumulation of radioactive cholesterol to both adrenals, in whom a unilateral adenoma was visualized by adrenal scintigraphy repeated after suppression of the cortisol production with dexamethasone. The inability of dexamethasone to suppress the accumulation of radioactivity in the adrenals in case 4 could mean that the primary aldosteronism in this patient was due to bilateral adrenocortical hyperplasia. The duration and timing of dexamethasone administration in relation to the injection of radioactive cholesterol could however be a factor of importance. As adrenal radioactivity usually becomes visible from the third day after injection of ¹³¹I-cholesterol and is maximal on the 6th or 7th day dexamethasone should probably be given 2 days before and at least 5–6 days after injection of the isotope to assure adequate suppression of the cortisol production. It is our opinion that until further experience is gained with scintigraphy after dexamethasone suppression catheterization with determination of plasma concentrations of aldosterone and cortisol from both adrenal veins combined with adrenal venography should be performed preoperatively in all patients with primary aldosteronism in whom adrenal scintigraphy is unable to localize a unilateral tumour even when performed during administration of dexamethasone. Possibly the diagnostic accuracy of adrenal scintigraphy could also be improved by adopting more objective methods for quantitation of the adrenal radioactivity.

Presumably the localization of an adrenal tumour as well as its differentiation from bilateral adrenocortical hyperplasia would be more accurate in patients with Cushing's syndrome. In bilateral hyperplasia a symmetrical concentration of radioactivity in both adrenals was to be expected. In the tumour cases the radioactivity is supposed to accumulate predominantly in the tumour, the function of the contralateral adrenal being suppressed. It is difficult to see how these assumptions could be wrong when the glucocorticoid excess is due to bilateral adrenocortical hyperplasia. The adrenal scintigraphy in case 5 the only patient in this cate-

gory we have examined, is in agreement with this as are the results reported by Lieberman et al (13)

In the two patients with Cushing s syndrome caused by an adrenocortical adenoma, the heavy concentration of radioactivity to one adrenal left no doubt of the tumour side In addition to the diagnostic value, the lack of accumulation of radioactivity in the contralateral adrenal implying a suppressed function also predicted that postoperative substitution therapy with corticosteroids would be necessary

So far our presuppositions hold true In the patient with adrenocortical carcinoma, however there was no scintigraphically detectable radioactivity in the large hormone-secreting tumour A slight and delayed accumulation of [131]I-cholesterol in the contralateral atrophic gland was misinterpreted leading to exploration on the wrong side. The reason why radioactive cholesterol was not concentrated in the tumour probably was a high cholesterol turnover with increased synthesis and secretion of corticosteroid hormones and their precursors The high cholesterol turnover was indicated not only by increased concentration of hormones in blood and urine but also by the finding that most of the cholesterol in the tumour was present as free non-esterified cholesterol. These observations—no accumulation of radioactivity in the adrenocortical tumour and suppression of the contralateral gland in two patients with adrenocortical carcinoma and one with adrenocortical adenoma—have also been made by others (2 13) and are bably typical findings in tumours with a high ocrine activity Such a scintigraphical pattern esents a diagnostic pitfall and radiological ethods will have to be adopted for correct side localization of the tumour

That the method is also applicable for the localization of phaeochromocytomas is demonstrated by case 10 The lack of radioactivity on the tumour side was due to compression of the adrenocortical cortex by the phaeochromocytoma. Identical findings in two patients with phaeochromocytoma have been reported by Anderson and Beierwaltes (2).

The visualization of adrenal remnants or ectopic adrenocortical tissue represents another problem not resolved by radiographical methods. Theoretically scintigraphy should be an ideal method and successful localization of adrenal remnants after 'total adrenalectomy for adrenocortical hyperplasia has been demonstrated (2, 13). Unfortunately in our patient with relapse of Cushing s syndrome after bilateral adrenalectomy (no 9) we did not have the same success The uptake of radioactive cholesterol in an ovarian corpus luteum cyst was mistaken for radioactivity in ectopic adrenocortical tissue Since the ovarian hormones are synthetized from cholesterol a concentration of circulating cholesterol to the ovaries is not surprising. The important question which still remains to be settled is whether accumulation of [131]I-cholesterol in the gonads regularly takes place and if it could be teratogenous in fertile patients. The radiation doses to ovaries and testes given by Beierwaltes et al. (5) may seem unimportant, but further studies are needed as the calculations are based on observations in only one patient. The inability to detect any radioactivity in the genital regions in two men and five women in whom we later performed adrenal scintigraphy could indicate that the risk is negligible. We prefer however to administer [131]I cholesterol early in the follicular phase in menstruating women and to perform the examination only in patients in whom the actual diagnosis is established clinically and by hormone assays.

In conclusion, we consider adrenal scintigraphy with [131]I 19-cholesterol to be a valuable method which in most cases of Cushing s syndrome will localize an adrenocortical tumour and distinguish tumour from bilateral adrenocortical hyperplasia. In primary aldosteronism the diagnostic accuracy of the method is less but may possibly be improved when performed under dexamethasone suppression and with more exact measurement of the radioactivity in each adrenal When no side difference is found determinations of hormone concentrations in both adrenal veins combined with adrenal phlebography should be performed

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BONE MASS IN OBESE SUBJECTS

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Abstract The inner and outer diameters of the cortex have been measured in 32 obese subjects i the middle of the second metacarpal bone and in the proximal part of radius. The results, which were compared with an age-matched control group showed that the obese subjects had on an average an 11% larger cortical area than the controls ($p<0.05$). The increased cortical area was caused by the greater outer diameter of the measured bones. There were no significant differences in inner diameters between the groups. The inner diameters increased with age in the same way in both obese and control persons, indicating that the former are not protected against osteoporosis in the form of endosteal resorption.

The question as to whether obese subjects, concomitantly with a hyperplasia or hypertrophy of the adipose tissue also have a similar change in the skeleton was raised in an earlier publication (4). On the basis of observations in four obese subjects in that study with the X-ray spectrophotometric method, a much higher bone mineral mass content was found than in the controls.

In order to elucidate whether obese persons in general have a high bone mass and if this could be caused by an increased periosteal apposition or a decreased endosteal resorption, the cortical dimensions in the second metacarpal bone and the proximal part of radius were measured.

MATERIAL AND METHODS

The obese subjects (22 women and 10 men) were all out patients, either on the waiting list for treatment at the Surgical Department, or operated upon with an intestinal shunt. Apart from their obesity they were in good general condition and had no other known metabolic disorder. They had undergone general physical examination, including chest X-ray and routine laboratory blood tests.

The control group was age-matched and drawn at random from the Stockholm population. The age distribution of the material measured was as follows. Obese women, age 30–34 $n=8$ age 45–49 $=8$ age 55–59 $=6$. Obese men, age 33–59 $=10$ Control women, age 30–34 $=8$, age 45–49 $=8$ age 55–59 $=6$ Control men, age 33–59 $=10$.

The measurements of bone dimensions were made at skeletal X-ray The inner and outer diameters of the cortex were measured in the middle of the second metacarpal bone (1) and in the proximal part of radius (8). The cortical area was calculated according to the formula $\pi(D^2-d^2)/4$ where D = outer diameter and d = inner diameter

The precision of the radiological appraisal, which was determined by replicate determinations, was for the outer diameters of the metacarpal bone and radius 1.9 and 3.1% respectively and for the inner diameters 5.2 and 5.8% respectively (5) When testing the hypothesis that obese subjects do not have more bone than controls, one-sided paired t-tests were used.

RESULTS

The obese subjects were not significantly taller than the controls ($p>0.05$) mean body height 166 cm (S.D 6) and 166 cm (S.D 6) respectively Mean body weights were 136 kg (S.D 22) and 63 kg (S.D 9), respectively which is a highly significant difference ($p<0.001$).

The obese patients had a significantly larger cortical area than the controls ($p<0.05$). The difference between the groups was caused mainly by greater outer diameter of the measured bones (Table I)

The groups displayed no significant differences in

Table I *Dimensions of the second metacarpal bone and proximal part of radius for 22 obese and 22 control females and for 10 obese and 10 control males*

The deviation for the obese patients is expressed as percentage of the mean values for the controls and the *t*-value is calculated for the means of the two samples. One-sided test

	Mean dimensions		Deviation		
	Obese	Control	(%)	*t*-value	*p*-value
Cortical area (mm²)					
Metacarpal					
Females	46	41	+12	2.1	<0.05
Males	61	53	+15	2.4	<0.05
Radius					
Females	91	84	+8	1.9	<0.05
Males	126	111	+14	1.9	<0.05
Outer diameter (mm)					
Metacarpal					
Females	8.3	8.0	+4	1.9	<0.05
Males	9.9	9.3	+6	1.8	<0.05
Radius					
Females	13.3	12.5	+6	2.5	<0.05
Males	15.1	14.6	+3	1.0	>0.05
Inner diameter (mm)					
Metacarpal					
Females	3.2	3.2	0	0.3	>0.05
Males	4.3	4.4	−2	0.1	>0.05
Radius					
Females	7.7	7.1	+8	1.9	<0.05
Males	8.2	8.3	−1	0.3	>0.05

inner diameter (Table I) which increased similarly with age (Table II).

There were no significant correlations between cortical area and the following clinical variables: height, blood calcium concentration or alkaline [illegible] in serum for the obese subjects (p>0.05).

DISCUSSION

The obese subjects had on an average an 11% larger cortical area than the controls ($p<0.05$). This is in agreement with our earlier study (4) in which we found, on an average, an 8% higher bone mineral content of the shafts of radius and ulna in obese subjects.

Table II *Inner and outer diameters of the second metacarpal bone and proximal part of radius for different age groups of women*

The parameters are expressed as percentage of the mean for the youngest group

	Obese women			Controls		
Age group (y.)	30–34	45–49	55–59	30–34	45–49	55–59
Outer diameter						
Metacarpal	100	100	98	100	108	107
Radius	100	100	105	100	105	98
Inner diameter						
Metacarpal	100	110	128	100	131	142
Radius	100	99	107	100	109	101

Skeletal hyperplasia is much more pronounced in trabecular than in cortical parts of the skeleton, but skeletal X-ray is not at present a suitable method for quantification of bone in trabecular parts. The cortical parts were therefore measured instead Another advantage of the present method is that it permits precise observations of the outer and inner diameters of the cortical bones, the relationship of which is an estimate of the balance between periosteal apposition and endosteal resorption.

The high bone mass in obese subjects may be due to an increased apposition or a decreased bone resorption. In the present study the large cortical area in the obese subjects was mainly caused by a greater outer diameter of the measured bones (Table I). The fact that some of the obese patients had undergone a jejunoileal bypass operation which results in a malabsorption would only influence the results by yielding a reduced bone mass. This source of error is of no importance here because the obese subjects had a significantly larger cortical area.

As a sign of endosteal resorption the inner diameters increased with age in the same way although less markedly as in the controls (Table II). This indicates that the obese subjects are not protected against osteoporosis in the form of an elevated endosteal resorption with increasing age This is, however a cross-sectional study and further elucidation of this problem calls for a longitudinal study

Little is known about the genesis of the greater cortical area in obesity It may however be due to overnutrition, as was indicated in an experimental study by Hedhammar et al (6) who found that overfed dogs of all ages had greater cortical thickness than controls.

Arthrosis with hyperplasia is common in obese persons (7) and is usually attributed to an increased load from the body weight. The overnutritioned dogs, however also had more joint complications which in the obese subjects might be caused to some extent by overnutrition in childhood Furthermore osteoporotic subjects reportedly have a tendency to be underweight, which is of great interest for this study (9)

The relationship between obesity and skeletal hyperplasia can be explained theoretically by assuming an excessive calcitonin production, as this both inhibits lipolysis in adipose tissue (10) and favours apposition of calcium in bones. Overnutrition also increases the secretion of gastrin which in turn stimulates the secretion of calcitonin (2 3)

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Table I *Plasma concentrations and urinary excretion of inorganic pyrophosphate (PP_i) before and during treatment with a high phosphate intake*

	Plasma		Urine					
Days	PP (µM)	P (mM)	Vol (l)	PP (µM)	P (mM)	PP_i (µmoles/24 h)	P (mmoles/24 h)	PP_i (µM) P (mM)
Before treatment								
3			2 450	82.19	7.89	201 4	19.3	10.4
1	6.26	1.22	1.675	213.30	9.94	357.3	16.7	21.5
During treatment with a high phosphate intake								
2	5 78	1.53	1 950	180.10	27 43	351.2	53.5	6.6
8	4.80	1.59	2.175	159.50	18.26	346.9	39 7	8.7
15	4.86	1.04	1.800	194.30	23 45	349 7	42.21	8.3
22	6.27	1 62	2.150	112.50	17 71	241 9	38.1	6.4
34	6.49	1 19						
69	8 19	1.25						
89	8.46	1.22	2.550	163.34	21.91	416.52	55.87	7.5
125	9.21	1.35	2.460	322.08	48.18	792.32	118.52	6.7

METHODS

Blood was drawn by venipuncture as atraumatically as possible into 5 ml heparinized plastic vessels surrounded by ice. It is important that no coagulation occurs because release of PP from platelets is well known (15). The plasma was immediately separated by centrifugation in the cold and 5 volumes of ice-cold perchloric acid were added After standing for 10 min in the cold the precipitate was removed by centrifugation and the supernatant neutralized with ice-cold KOH This procedure has been found to give 100 % recovery of PP The urine was treated with perchloric acid in a similar way in order to remove any inorganic pyrophosphatase activity Such treatment of urine gave 100 % recovery of PP The analytical thod for PP determination has been described else ere (7) The P was measured by the method of Wahler Wollenberger (16).

CASE REPORT

Previous history

The patient is 53-year-old married woman. There is no family history of bone diseases but two brothers died in infancy from unknown causes. Her mother has old-age diabetes. One year old the patient was admitted to a sanatorium for one year because of "rickets She did not walk until two years old. She lost her permanent teeth because of "fragile enamel" at the age of 15 She was pregnant only once but had a spontaneous abortion.

At the age of 29 and 39 cysts were removed from her ovaries. After menopause at 45 she had oestrogen/androgen (Femovirin® 1 ml) injections every month because of climacteric symptoms. Since 40 she has often suffered from headache for which she has been taking up to 4 g aspirin a day

Actual bone and joint history

In 1960 the patient had Colles fracture of the left wrist after adequate trauma and with prompt healing. In 1966 she developed pains in her right foot. X-ray was normal but two years later several fractures of the metatarsal bones were seen. With the exception of one year—1970—when the patient was able to resume her work in a factory she gradually developed incapacitating pains in her feet, knees hips, low back and wrist, and successive fractures in the calcaneus processus styloideus of the ulna and radius, both tibiae and fibulae as well as pseudofractures of both femora. A general progressive halisteresis was observed and radiological and clinical signs of arthroses in both lower extremities, as well as calcification of the spinal ligaments.

Diagnosis

Blood examinations were not carried out until 1970 when a very low alkaline phosphatase activity was found Later in the same year a search for hyperparathyroidism was made with negative result. I 1973 hypophosphatasia was suspected because of the invariably subnormal alkaline phosphatase activities and the diagnosis was confirmed by the finding of a 24-hour urinary excretion of 244 µmoles phosphoethanolamine value far above normal (12). Traces of the same compound were found in plasma.

A bone biopsy taken in 1973 contained considerable amounts of uncalcified osteoid Alkaline phosphatase activity was normal in biopsy from the small intestine. Histochemical examination of granulocytes for alkaline phosphatase activity gave the very low score of 4 (NR 40–100)

Other laboratory investigations

The patient s height was 155 cm, weight 55 kg, BP 140/90. Total blood count was normal ESR 7 mm/h. Alkaline phosphatase in serum was 3–10 U/l (NR 13–38, modified

Bessey-Lowry)' isoenzyme determinations were not possible, the total activity being too low. Acid phosphatase was 7.1 U/l (2.7–10.5). Alanine aminotransferase, α-amylase, bilirubin and prothrombin were normal. Serum electrophoresis normal. In plasma the following concentrations were found: calcium 2.53 mM, phosphate 1.56 mM, sodium 142 mM, potassium 3.9 mM, standard bicarbonate 24.2 mM, and magnesium 0.83 mM. Serum creatinine was 0.09 mM, carbamide 7.8 mM, creatinine clearance 0.94 ml/sec (NR 1.6–2.0). A 24-hour urine contained 4.6 mmoles calcium and 17 mmoles phosphate. There was no albumin, blood or glucose in the urine, and microscopy was normal. Traces of blood were found in her feces; 9 mmoles lipid were excreted in a 24-hour feces sample.

Radiologically her chest, cranium and stomach appeared normal. Intravenous pyelograms were slightly contracted. ECGs were normal. Serum thyroxine was 95 nM. A calcium balance study showed normal results and measurements of mineral contents in bone showed low normal values.

Orthopedic treatment

At first, in 1967, when no bone or joint lesions had been found, the patient's pains were interpreted as muscular and treated with physical therapy. Later, when multiple fractures were found in her right foot, this was immobilized with good result. In 1972 the patient had pains in her left knee and crus for 4 months before a fracture was diagnosed. Even 4 months of immobilization did not result in healing. In March 1973, therefore, an osteosynthesis of the left tibia was performed. Increasing consolidation resulted, but one year later the fracture line was still visible. A somewhat similar course was observed for the right tibia. The fracture line was first seen in Nov. 1973 but as the patient hardly ever stood on her legs, but moved around using crutches or in a wheelchair, no further treatment was found necessary. However the fracture line became more and more distinct and an osteosynthesis of the right tibia was performed in May 1974.

Phosphate treatment

The patient has never received cortisone, calcium or vitamin D treatment.

A preparation of disodium hydrogen phosphate, 300 mg, and potassium dihydrogen phosphate, 200 mg, was used. The patient received 12 g of this compound a day, as 8 tablets three times a day, which corresponds to an increased phosphorus intake of 1.98 g/day.

Before onset of the high phosphate intake the patient was admitted to hospital and kept on a standard diet containing 1260 mg phosphorus per day in order to establish a pretreatment level of plasma PP.

The plasma concentration and the urinary excretion of PP were elevated before as well as during treatment (Table I). The normal concentration of PP in plasma is 1.92±0.092 μM (S.E.M., n=23) determined by the isotope derivative method. The excretion of PP in urine from volunteers of the same age as the patient has been found to be in the region of 30 μmoles/24 hours. This is in good agreement with the findings of Fleisch and Bisaz (4) whereas the plasma values are about half the magnitude reported by Russell et al. (14).

The plasma P concentration increased slightly during the high phosphate intake while the urinary excretion augmented to about three times the pretreatment value (Table I). The plasma PP concentration and the urinary excretion did not change. Consequently the PP/P ratio fell to about 7, which is still far above the ratio of approximately 0._ found in normal subjects (4).

DISCUSSION

More than 10 cases of adult hypophosphatasia have now been described (1, 9, 10, 11). In most of them the disease has been misinterpreted in early childhood as rickets. There is no sex preference, though the tendency of postmenopausal women to develop osteoporosis may provoke some latent cases. Typically there are symmetric fractures and pseudofractures of the lower extremities. Most of the reported adult cases had symmetrical pseudofractures of the femoral diaphyses. Progressive halisteresis has been noted in several cases as well as calcification of the spinal ligaments and chondrocalcinosis (11). In many respects, our patient had the characteristic syndrome.

Since Fleisch and Bisaz (5) showed that PP concentrations in the range 10^{-6}–10^{-} M effectively inhibited collagen-induced calcium phosphate precipitation in vitro, it is tempting to speculate that the abnormally high PP concentrations in the biological fluids in hypophosphatasic patients may prevent normal calcification. Russell (13) suggested that a high phosphate intake might lead to improvement in this disease. In a later paper (14), however, he stated (without documentation) that such treatment did not change PP concentrations in plasma and urine. This is in agreement with our results and may explain why a beneficial effect in our patient has been lacking so far. It has proved impossible to increase the PP excretion by loading with P. In normal subjects an increased P excretion is accompanied by an increased PP excretion, so that the PP/P ratio remains constant (4). The renal tubular transport of PP in our patient may have been saturated and this would explain why PP, circulating in an abnormally high concentration, cannot be removed from the body. In the cases reported by Bongiovanni et al. (2) and Guibaud and Larbre (8), the treated children showed significant clinical and radiological improvement. Bongiovanni et al. found a rise in urinary pyrophosphate, which

has not been confirmed by Guilbaud and Larbre or the present work. We did not find any improvement clinically or radiologically during the treatment which may be due to our patient's age.

The isotope derivative method used in the present study for measuring PP_i is specific and very sensitive (10^{-8} M can be measured in a sample volume of 50 μl with an S.D. less than 5%). More correct concentrations of PP_i in plasma from normal subjects (1.92±0.09? μM) and from a hypophosphatasic patient have now been established. The higher values for plasma PP both in normals and in hypophosphatasia reported by Russell et al. (*14*) must be due to a systemic error. Their conclusion that PP_i is invariably elevated in plasma and urine in this disease is confirmed by the present work. Silcox and McCarty (15) recently reported a PP concentration in plasma of 1.8±0.06 μM (S.E.M.) from 94 normal subjects. The essential step in their procedure is based upon a specific enzyme and is in very good agreement with our results.

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THE ACUTE EFFECT OF SODIUM CELLULOSE PHOSPHATE ON INTESTINAL ABSORPTION AND URINARY EXCRETION OF CALCIUM IN MAN

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Abstract. Intestinal ^{47}Ca absorption has been determined from blood and stool radioactivity after oral administration of the isotope in nine patients before and during cellulose phosphate treatment. Oral administration of 5 g cellulose phosphate concomitant with ^{47}Ca in 100 mg "carrier" calcium as $CaCl_2$ decreased ^{47}Ca absorption by 80% Cellulose phosphate, 5 g three times daily decreased urinary excretion of non-radioactive calcium by 47% Urinary magnesium excretion decreased by 47% whereas urinary phosphorus excretion increased by 67% Calcium and magnesium excretion in urine decreases because cellulose phosphate binds divalent cations within the GI tract. The increased phosphorus excretion is probably due to partial hydrolysi of the substance in the gut.

Hypercalciuria is present in a significant number of patients suffering from recurrent calcium-containing renal calculi and urine calcium may play an important role in stone formation (8 13 15). A reduction of urinary calcium excretion has therefore been attempted in idiopathic stone formers by several therapeutic measures such as low calcium diet (15 22) thiazide administration (24 25), oral inorganic phosphate (3) or cellulose phosphate (5 17 20). Inorganic soluble phosphate was used empirically Although it reduces urine calcium (3), the mechanism by which it affects stone formation may be more complex (15). The therapy seems rather ineffective (7) and can cause extraskeletal calcifications (6) due to absorption of phosphate Cellulose phosphate is on the other hand essentially unabsorbable. The substance is described as an ion exchange resin with particular affinity for divalent cations (17). When given orally it is supposed to act by binding dietary calcium and thereby decreasing its intestinal absorption and urinary excretion. In metabolic balance studies cellulose phosphate has been found to increase faecal and decrease urinary calcium excretion in some small series (5 14 21) More direct information on its effect on calcium absorption is scanty (17 20)

In the present study the intestinal absorption of ^{47}Ca has been determined in patients from blood and stool radioactivity after oral administration of the isotope before and during treatment with cellulose phosphate

MATERIAL AND METHODS

The material comprised nine patients. Six suffered from recurrent radio-opaque renal stones, one had sarcoidosis one hyperparathyroidism and one duodenal ulcer

Calcium absorption was measured as described by A loh et al. (2) with some modifications About 10 µCi ^{47}Ca as $^{47}CaCl_2$ in 100 mg Ca carrier as $CaCl_2$, was given orally in 154 mM NaCl containing 4 g polyethylene glycol (PEG MW 4 000). One capsule of carmine red (0.5 g) was taken with the calcium solution. The patients were allowed free diet but fasted for 12 hours before and 2 hours after calcium administration. Two absorption tests were carried out in each individual with an interval of 2 weeks to 3 months. In one of the two tests 5 g sodium cellulose phosphate (Calcisorb® ICN) was given orally immediately after the calcium dosage During the following two days 5 g cellulose phosphate was given 3 times daily with meals.

Blood samples were drawn 1/2, 1 2 3 and 4 hours after administration of the isotope. Urine was collected for 2 days and faeces until they were no longer coloured red by carmine

Gamma radioactivity of ^{47}Ca in serum and faeces was counted in the Hormone and Isotope Laboratory Aker Hospital in 2 ml samples in a gamma spectrometer with 3 inch crystal A 2 mm lead screen was placed around the sample tube to screen out radiation from ^{47}Sc (1), the daughter isotope of ^{47}Ca which is not absorbed from the GI tract (16).

Faeces were pooled, diluted with water weighed and

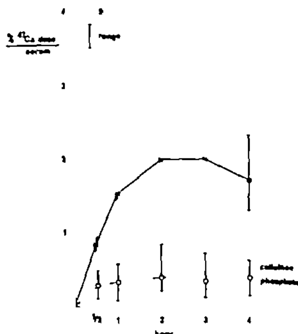


Fig. 1. Serum concentration of ^{47}Ca (% of ^{47}Ca dose/l serum) after oral administration of the isotope before (●) and after (O) administration of cellulose phosphate (means and ranges).

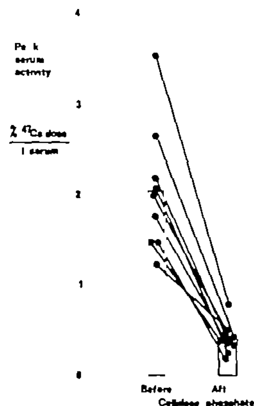


Fig. 2. Peak serum concentration of ^{47}Ca (% of ^{47}Ca dose/l serum) after oral administration of the isotope. Individual values combined before and after administration of cellulose phosphate. The columns indicate means.

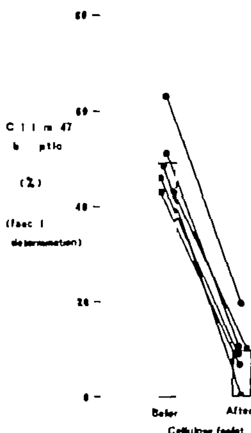


Fig. 3. Net calcium absorption (%) calculated as the difference between the amount of ^{47}Ca administered and the amount of the isotope recovered in faeces corrected for loss. Individual values combined before and after administration of cellulose phosphate. The columns indicate means.

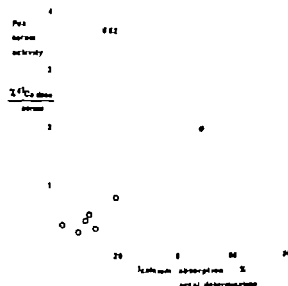


Fig. 4. Relationship between ^{47}Ca absorption determined by stool and serum radioactivity. ● = values before, O = after administration of cellulose phosphate.

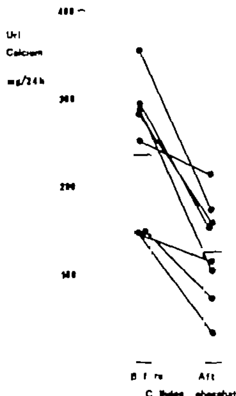


Fig. 5. Urinary excretion of non-radioactive calcium before and during cellulose phosphate treatment, 5 g three times daily. Individual values combined. Columns indicate means.

homogenized (11) before counting of ^{47}Ca in weighed samples. PEG was determined according to *Hydén (10)*. PEG served as an inert unabsorbable marker to allow correc tion for incomplete recovery of ^{47}Ca excreted in stools, assuming similar percentage loss of unabsorbed ^{47}Ca and PEG. The assumption was tested in preliminary studies by determination of ^{47}Ca and PEG in daily stool specimens. It was found that the GI transit times of PEG and ^{47}Ca were approximately similar. The percentage loss due to incomplete collection should therefore be approximately similar for PEG and ^{47}Ca. Net absorption of ^{47}Ca was then calculated as the difference between the amounts of ^{47}Ca administered and the amounts recovered in faeces corrected for loss.

By using carmine red and collecting the red-coloured faeces, sufficient amounts of ^{47}Ca and PEG were recovered for accurate analyses without prolonging the faecal collection unnecessary. Mean recovery of PEG in the 6 patients who completed the faecal collection was 83 3% (range 56...–112.0%). With the present method duplicate determinations of PEG on separate days in each of 23 faecal samples yielded a coefficient of variation of 3 7%.

Ca, Mg, and P in urine were determined in our Central Laboratory by routine methods, i.e. Ca and Mg by atomic absorption and P colourimetrically with molybdat

RESULTS

Without cellulose phophate the maximal concentration of ^{47}Ca in serum was found 2 3 and 4 hours after administration of the isotope in 4 3 and 2 of the patients respectively. The highest serum ^{47}Ca levels were observed in the patient with sarcoidosis and the patient with hyperparathyroidism. After administration of cellulose phosphate the serum concentration of ^{47}Ca was markedly reduced in all the patients. The mean values with and without cellulose phosphate in relation to the ^{47}Ca intake are shown in Fig. 1. Peak serum concentration of ^{47}Ca, i.e. the mean of the maximal and the preceding concentration recording was reduced by cellulose phosphate on an average by 79 4% (range 59 0–88.3%) (Fig. 2).

^{47}Ca absorption determined by stool radioactivity was similarly reduced by cellulose phosphate (Fig 3) on an average by 81.6% (range 69 0–100%). ^{47}Ca absorption determined by serum and stool radioactivity showed a good correlation ($r=0.92$) (Fig. 4).

The urinary excretion of non-radioactive calcium decreased in each subject during cellulose phosphate treatment (Fig. 5). The reduction averaged 46.8% (range 15.2–77.2%). Mean magnesium excretion in urine decreased by 47% whereas mean excretion of inorganic phosphorus increased by 67% (Table I). The changes were found in each subject and were statistically highly significant ($p<0.001$)

Table I. *Magnesium and inorganic phosphorus in urine before (B) and during treatment (D) with sodium cellulose phosphate*

Case no.	Magnesium (mEq/24 h)		Phosphorus (mg/24 h)	
	B	D	B	D
1			713	797
2	8.2	4.6	1 001	1 133
3	8.5	4.5	913	1 343
4	4 4	1.8	480	811
5	5 7	5.0	730	1 258
6	15.2	12.4	957	1 467
7	8.2	6 5	1 307	1 929
8	5 3	2 5	705	1 251
Mean	7 9	5.3	851	1 249
S.D	3 6	3.5	290	364

DISCUSSION

The estimation of intestinal calcium absorption by serum radioactivity after oral administration of ^{47}Ca suffers from the disadvantage that the serum level achieved depends on the concomitant removal of the isotope from the blood mainly into bone and urine. A more valid estimate of ^{47}Ca absorption could be obtained by determining the amount of unabsorbed ^{47}Ca excreted in faeces provided the faecal ^{47}Ca could be completely recovered. This is however practically impossible and an unabsorbable marker has to be used so that corrections can be made for incomplete collection. PEG is widely used as an inert unabsorbable marker in the GI tract, but does not appear to have been employed in clinical isotopic calcium absorption studies. It passes through the GI tract without being absorbed or split and can be determined with reasonable accuracy in biological material by a turbidimetric reaction (9 10 12). In the present study the marker was found to be applicable for quantitating faecal loss of ^{47}Ca, and the ^{47}Ca absorption determined by the stool radioactivity correlated well with the serum radioactivity.

The present results show that 5 g sodium cellulose phosphate effectively inhibited the intestinal absorption of ^{47}Ca when the isotope was given with 100 mg of carrier calcium as calcium chloride. The dietary calcium load is of course appreciably higher. Our normal hospital diet contains about 1100 mg calcium daily. This larger calcium load surpass the binding capacity of cellulose making the inhibition insignificant. The reduction of non-radioactive calcium excretion in urine in the present and other studies (3 5 14 21) suggests however that 5 g cellulose phosphate three times daily significantly decreases the absorption of dietary calcium as well.

Since cellulose phosphate has the property of binding divalent cations it also binds dietary magnesium (17). The urinary excretion of magnesium was consistently decreased in the present study during cellulose phosphate treatment. Magnesium therefore has to be supplemented when cellulose phosphate is given to patients for long periods (18). The increased phosphorus excretion in urine probably means that the substance is partially hydrolyzed in the gut part of its phosphate content being absorbed (5 18). Both magnesium and phosphate in urine may influence stone formation (15) but to what extent cellulose phosphate affects stone formation by such mechanisms is not known.

The dependence of urinary calcium on dietary calcium (22) as well as the increased calcium absorption found in some patients with hypercalciuria (4 8, 19) and recurrent urinary calculi (14) suggest that the intestinal absorption of calcium may play an important role in idiopathic stone formation (15). Treatment with substances lowering calcium absorption seems therefore logical. Recently Pak et al (18) reported that cellulose phosphate was highly effective in preventing recurrent renal calculi during long-term treatment. Their findings may support the view that the initiation of stone formation is related to the precipitation of a nidus of calcium phosphate (17 18 19 20) and that reduction of urinary calcium excretion by decreasing intestinal calcium absorption will reduce the propensity to stone formation. The treatment with cellulose phosphate is however somewhat inconvenient as the rather large volume of insoluble cellulose phosphate must be taken about three times daily in addition to magnesium supplement. The treatment has been found to be notably free of side-effects (18). Both hypocalcemia, hypomagnesemia and increased phosphate intake may lead to secondary hyperparathyroidism (23). Pak et al (18) found however that the parathyroid hormone concentration in blood was within the normal range during cellulose phosphate treatment. It is not known whether the calcium depletion will lead to a rebound increase in calcium absorption when cellulose phosphate treatment is stopped or interrupted.

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SERUM CALCITONIN RESPONSE TO INDUCED HYPERCALCEMIA

A Diagnostic Aid in Early Occult Medullary Thyroid Carcinoma

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Abstract. The rise in serum calcitonin (Δ-CT_{max}) has been measured during hypercalcemia induced by i.v. infusion of calcium gluconate. This calcium infusion test was used in a prospective screening for medullary carcinoma of the thyroid (MCT) in 4 families with Sipple's syndrome as well as in 3 sporadic cases of MCT. In 16 normal controls Δ-CT_{max} was -0.4–$+0.5$ ng/ml (mean ± 2 S.D.). Δ-CT_{max} was normal in 2 patients with chronic hypocalcemia. In all 14 MCT patients Δ-CT_{max} was markedly higher (min–max 2.2–630 ng/ml), i.e. no false negatives were found. However, in these cases, the diagnosis was already evident from basal serum calcitonin (S-CT), which up to now has been our most sensitive diagnostic technique for MCT. 19 first-degree relatives of patients with Sipple's syndrome presented no signs of MCT. In 14 of these Δ-CT_{max} was normal ("healthy relatives"), but in 5 it was slightly elevated, intermediate between the controls and the MCT patients. These 5 borderline cases were more sharply delineated from normal by Δ-CT_{max} than by S-CT. Thus our calcium infusion test seems to be the most sensitive method for early diagnosis of occult MCT. We recommend the calcium infusion test for: (*a*) screening for MCT in all Sipple relatives with normal or only slightly elevated basal S-CT; (*b*) postoperative control in both sporadic and hereditary MCT; (*c*) investigation of supposed non-MCT tumours with calcitonin production.

Determination of basal serum calcitonin (S-CT) with a specific and sensitive radioimmunoassay is very useful in the diagnosis of medullary carcinoma of the thyroid (MCT) (1, 4, 17). It is superior to conventional methods (clinical examination and thyroid scintiscan), fine needle biopsy (25) and determination of serum diamine oxidase activity (histaminase E.C. 1.4.3.6.) (27).

Both in normal human C cells and in medullary carcinoma cells, calcitonin (CT) secretion is stimulated by hypercalcemia (17, 23).

In patients with very small tumours the basal level of S-CT may be normal or close to the upper normal limit, e.g. in early cases and after non-radical surgery. In these patients determination of the S-CT response to induced hypercalcemia might provide us with an even more sensitive method that would disclose occult, i.e. asymptomatic tumours. The purpose of this investigation was to evaluate this hypothesis.

MATERIAL

The patient material comprised three cases of sporadic MCT (S 1–3) and 30 members of four separate families (families I–IV) with Sipple's syndrome. This is a hereditary syndrome with MCT usually combined with bilateral phaeochromocytomas (21, 27), which is transmitted as an autosomal dominant trait.

The Sipple families were screened prospectively for these hereditary tumours. Our screening program for MCT includes 1) careful clinical examination, 2) thyroid scintiscan using ^{131}I and/or ^{99m}Tc, 3) fine needle aspiration biopsy of all palpable thyroid nodules and/or lymph nodes of the neck, 4) determination of serum diamine oxidase activity (histaminase E.C. 1.4.3.6.) and 5) determination of S-CT using our radioimmunoassay method for human S-CT (1), both (*a*) basal level and (*b*) the response to induced hypercalcemia, which is the subject of this report.

This screening program revealed 8 new cases of MCT (patients I 1–3, II 1–3, III 1–2). All Sipple patients except one (II 2) as well as the sporadic cases (Table I) had their MCT diagnosis verified by surgery and histopathology using the criteria of Hazard et al. (11) and Williams et al. (30). Patient II 2 refused surgery, but his Sipple's syndrome was regarded as indirectly verified as he was genetically proved carrier of the Sipple genome. He also had raised basal S-CT as well as bilateral phaeochromocytomas, verified by adrenal angiography and high urine excretion of VMA and catecholamines.

Three Sipple patients (III 3–5), earlier operated upon for MCT, were examined for occult metastases. They had no clinical signs of recurrence. Six patients were examined both before and after surgery for MCT (Table II).

Controls. Sixteen healthy volunteers of either sex, 15–60 years, without clinical or laboratory signs

Table I. S-CT respons to calcium infusion (Δ-CT$_d$) in 14 patients with MCT

Pat. no.	Sex	Age (y)	S-CT at 0	Δ-CT$_{60}$	Δ-CT$_{240}$
I.1	♂	57	77.3	10.9	
I.2	♀	54	5 1	1 7	4.2
I.3	♀	22	2.7	0.7	2.2
II.1	♂	58	5 2	5.6	9.5
II.2	♂	59	2.4	1 4	4.2
II.3	♂	33	4.9	4 4	14 4
III.1	♂	21	0	3.1	7.5
III.2	♀	21	2.7	1 0	7 4
			2.5	3.2	8.[illegible]
III.3	♀	48	3 4	1.8	12.9
III.4	♂	57	5 1	3.2	4 4
III.5	♂	55	13 4	0 6	10.6
S.1	♂	71	980	250	630
S.2	♀	78	6.7	4.9	12.1
S.3	♀	67	1.6	0 9	13 4
Upper reference limit (ng/ml)			1 04	0.43	0.90

Earlier operated on for MCT

ordered thyroid or calcium metabolism served a controls. Furthermore we examined two women (patients H.1 and H.2) with long-standing hypocalcemia due to intestinal malabsorption. Patient H 1 was 47 years old had an intestinal radiation injury after radiotherapy against a carcinoma of the ovary and had been treated with vitamin D although insufficiently. Patient H.2 wa 34 years old and probably had gluten-sensitive enteropathy. Both had serum calcium (S-Ca) levels around 3 6–3 9 mEq/l (Table III).

METHODS

Serum calcitonin. The radioimmunoassay technique has been described in detail in a previous paper (1). S-CT levels expressed as ng/ml. Reference values 0.36–1 04 ng/ml.

um calcium. S-Ca wa determined by flame emission metry

Calcium inf sion test. Transient hypercalcemia wa induced by infusing 3 75 mmol=7.5 mEq=15 mg Ca^{++}/kg b wt. calcium gluconate in 1000 ml 10% invertose solution during 4 hours. All subjects were normocalcemic before the infusion and had normal renal function. All except patient II 2 were fasting The infusion induced a rise in S-Ca of 1 43±2×0.56 mEq/l (mean ±2 S.D) At the end of the infusion S-Ca wa 6.25±.×0.44 mEq/l (mean ±2 S.D) MCT patients did not differ from the control in th respect.

S-CT was analysed in peripheral venous blood taken at 0′ 60′ and 40′ S-CT at 0′ is the arithmetic mean of two separate samples taken t −5′ and just before the start of the infusion. Zero time (0′) is the start of the infusion, usually at about 10 a.m. In seven patients additional samples were taken t 30′ 90′ 120′ and 180′ The expression "normal range" for Δ-CT$_{60}$=mean ±2 S.D of healthy control The S-CT rise during induced hypercalcemia at t min after the start, zero time, is denoted Δ-CT

RESULTS

Healthy controls

In the 16 healthy controls Δ-CT$_{60}$ was small, normal range −0.2−+0.5 ng/ml (mean ±2 S D =0.14±2×0 18 ng/ml, $p<0.01$ for the difference from zero) (Table III Fig. 1)

MCT patients

All 14 patients with MCT (11 hereditary and 3 sporadic cases) had elevated levels of basal S-CT and significantly higher Δ-CT$_{240}$ than the control group The range of Δ-CT$_{240}$ was 2.2–630 ng/ml (Table I Figs 1 and 2)

In order to study the reproducibility of the results of calcium infusion test one patient (III.2) was examined twice at an interval of 2 months (Table I). The results differed very little

In 7 MCT patients S-CT analyses were performed at closer intervals at 0′ 30 60′ 90 120′ and 40′ In 4 of these patients the rise in S-CT was not uniform over time but showed an initial rise during the first hour (phase 1) then a flatter (phase 2) and finally a steeper rise again (phase 3) (Fig. 3).

In 2 of the 14 MCT patients (I.2 and III.2) determinations of basal S-CT and Δ-CT$_{240}$ were the sole indications for surgery all other examinations being negative The diagnosis of MCT was confirmed in both cases

In 6 MCT patients calcium infusion was performed both preoperatively and within one month postoperatively in order to check the need for extended surgery (Table II) All but one (case II.1) showed a normal response to calcium infusion postoperatively (Fig. 4) This was interpreted as the result of radical surgery which was not contradicted by histologic evidence. These 5 patients have been

Table II. Pre- and postoperative levels of basal S-CT and Δ-CT$_{240}$ (ng/ml) during calcium infusion in 6 patients operated on for MCT

	Preoperative		Postoperative	
Pat. no.	Basal S-CT	Δ-CT$_{240}$	Basal S-CT	Δ-CT$_{240}$
I.3	2.7	2.2	0.2	0.3
II 1	5.2	9.5	2.2	3 7
II.3	4 9	14 4	0.9	0
III.1	2.0	7.5	0.5	0.1
III.2	[illegible]7	7 4	0.5	0
S.2	6.7	12.1	0.9	0 4

Table III. *S-CT response (Δ-CT$_x$) (ng/ml) to calcium infusion (calcium gluconate 3.75 mmol=7.5 mEq=15 mg Ca^{++}/kg b. wt./4 h) in (a) 16 healthy controls (b) 2 patients with chronic hypocalcemia and (c) 14 apparently healthy first-degree relatives of Sipple patients*

	S-CT at 0'	Δ-CT$_{60}$	Δ-CT$_{240}$
(a) Mean ±2 S.D.	0.69±2×0.16	0.07±2×0.18	0.14±2×0.18
Range	0.36–1.01	−0.29–+0.43	−0.23–+0.50
(b) Pat. H.1	0.8	−0.1	−0.3
Pat. H.2	0.7	0	0.2
(c) Mean ±2 S.D.	0.70±2×0.16	0.00±2×0.08	0.04±2×0.19
Range	0.38–1.02	−0.16–+0.16	−0.34–+0.42

followed regularly (0–4 years) with clinical examinations and determinations of basal S-CT. They have all remained healthy with normal S-CT levels.

However, in case II 1 neither basal S-CT nor Δ-CT$_{240}$ became quite normal after surgery (Fig. 4). This was interpreted as consistent with the presence of remaining tumour tissue, although there was sufficient radicality of the surgical specimen according to histologic evaluation. Follow-up during two years has shown steadily rising levels of basal S-CT and Δ-CT$_{240}$ up to 14 and 44 ng/ml respectively. Clinical, roentgenological and scintigraphic examinations are normal. Selective venous catheterization with sampling for S-CT analysis shows very high S-CT levels in the right superior thyroid, both jugular, azygos and hepatic veins. We interpret these biochemical findings as indicating the presence of multiple metastases with progressive growth.

Three hereditary cases (III 3–5) had previously been treated for MCT with bilateral neck lymph node metastases. They had all been operated on with total thyroidectomy, bilateral "neck dissection" and postoperative external radiotherapy. Follow-ups during 10, 8 and 6 years, respectively, had not revealed clinical signs of recurrence. Thus the raised levels of basal S-CT, confirmed by definitely abnormal Δ-CT$_{240}$ in all three patients, were surprising (Table I). Also in these cases the S-CT levels were interpreted as indicating occult metastases obviously growing very slowly. The presence of metastases has not been verified by other means, the patients being unwilling to undergo further examinations.

Healthy relatives

Fourteen first-degree relatives of Sipple patients, having neither palpable thyroid abnormalities nor any clinical signs of pheochromocytoma, were found to have normal basal levels of S-CT (mean ±2 S.D.=0.70±2×0.16) and Δ-CT$_{240}$ within the normal range (range −0.2–+0.5 ng/ml, mean ±2 S.D.=0.04±2×0.19). These individuals were provisionally considered "healthy" as these levels coincide with those of our healthy controls (Table III, Fig. 1).

Borderline cases

Another 5 first-degree relatives, also lacking all clinical signs of the tumours of Sipple's syndrome, had slightly elevated Δ-CT$_{240}$ levels within the range +0.9–+1.7 ng/ml. With regard to Δ-CT$_{240}$ these rela-

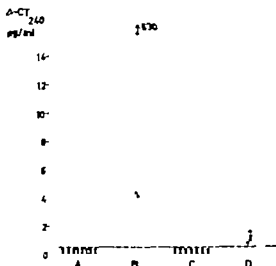


Fig. 1. S-CT response (Δ-CT$_{240}$) to induced hypercalcemia (i.v. infusion of calcium gluconate, 3.75 mmol=7.5 mEq=15 mg Ca^{++}/kg b. wt./4 h). *A*=16 healthy controls, *B*=13 cases of verified MCT, *C*=14 apparently healthy first-degree relatives of Sipple patients, *D*=5 Sipple relatives classified as borderline cases.

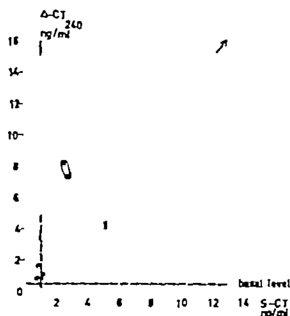


Fig 2 S-CT response to induced hypercalcemia related to basal S-CT in 13 cases of verified MCT (×) and in 5 borderline cases (●). Patient III... (encircled) was tested twice Area within broken lines represents upper normal range (mean +2 S.D) for S-CT and Δ-CT_{240}.

tives were classified as borderline cases the range of their S-CT response being intermediate between the upper border for the healthy controls and the lowest response of the verified MCT cases (0.5 and 2.2 ng/ml respectively) (Table IV*a* Figs 1 and 2).

They were reexamined with another calcium infusion test about one year after the first investigation able IV *b*) At the second examination patients II 4 III 6 had developed both a higher basal S-CT a much higher Δ-CT_{240}. On the basis of our *ience of verified MCT patients, we consider* this progress to be consistent with MCT It should be noted that the first basal S-CT in patient II 4 was also slightly elevated 1 9 ng/ml Both patients although still without palpable tumours will be operated on with a total thyroidectomy In the others Δ-CT_{240} is still slightly elevated most strikingly in patient III 8

Control patients with chronic hypocalcemia

These patients were examined in order to reveal possible abnormal stores of unused CT in the C cells, which might cause an elevated S-CT response to a calcium infusion test. However Δ-CT up to 5 hours were within normal range Thus chronic hypocalcemia does not seem to cause false positive results of Δ-CT as regards the diagnosis of MCT (Table III)

Determination of Δ-CT_{60}

As seen in Table I even Δ-CT_{60} was able to discriminate all patients with verified MCT from healthy controls. But the difference from normal is still more evident if the calcium infusion is continued for 4 hours, whether Δ-CT_{240} is expressed in absolute concentrations or in S D from normal mean.

DISCUSSION

Cancer diagnosis by a simple blood analysis has been the distant goal of much research in recent years, although as a whole with little success A few tumours however may now be diagnosed in this way Multiple myeloma cells produce excessive amounts of abnormal immunoglobulins hepatic carcinoma may synthesize α-fetoprotein and carcinoma of the colon secretes carcino-embryonic antigen.

Some endocrine tumours, benign and malignant, can be revealed by blood and urine analyses showing excessive amounts of the respective hormones or degradation products hereof

MCT is an example of such a neoplasm composed of endocrine cells with hormone synthesis. In spite of their malignant character they have retained the capacity to store and in response to hypercalcemia, release the hormone CT just like normal C cells (17 23)

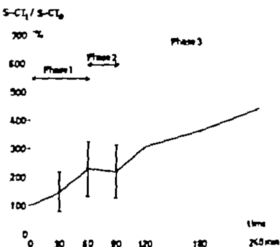


Fig 3 Relative S-CT rise during induced hypercalcemia in 7 MCT patients (mean ±S.D). S-CT at time t = S-CT_{basal} + Δ-CT

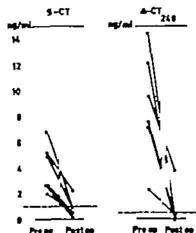


Fig 4 Pre- and postoperative levels of basal S-CT and Δ-CT$_{240}$ in 6 patients operated on for MCT —=upper normal reference limit.

It has been known since 1968 that MCT contains large amounts of CT and that the serum concentration of this hormone in MCT patients is very high compared with that of healthy people. Since 1969 a number of laboratories in different countries have been able to determine S-CT in MCT patients using more or less sensitive and specific radioimmunoassays (1 2, 3 9 12, 23 26) although only a few can measure normal levels (1 12 23 26) This has opened new efficient ways of diagnosing MCT earlier and more safely through a simple analysis of venous blood Thus one goal of diagnostic research has been reached for this particular tumour

MCT is unusual in that it may occur in a hereditary form the gene of this so-called Sipple syndrome being transmitted as an autosomal dominant trait. Thus, 50% of the children of an affected parent are expected to develop this tumour usually in combination with bilateral pheochromocytomas A number of these families have been reported in recent years (15 18 21 4). They can be considered as high risk populations for these tumours and their members should be screened regularly in order to diagnose and treat tumours as early as possible

Unfortunately *we have to wait for the neoplasm to develop before it can be diagnosed and treated* Even studies with advanced modern technique could not demonstrate chromosomal changes in Sipple s syndrome, which would help us to find the Sipple genome carrier at an earlier stage (14 20).

The introduction of S-CT determination as a routine diagnostic screening method in these families will probably radically improve the prognosis of tumour-bearing individuals as with earlier diagnosis radical treatment can be achieved before the development of metastases

During 1970–73 we screened three separate families with Sipple s syndrome and in 1974 we started screening a fourth Swedish family The clinical details will be presented in a separate paper Our family studies have proceeded parallel to those in Boston (18). Both studies show that *basal S-CT* is raised in *most* or all patients with verified MCT although very little in the earliest cases. These results are also in accordance with those published by Deftos (4)

Using the determination of *basal S-CT* we have been able to diagnose early multiple tumours of pinpoint to ricegrain size (patients III 1 and III_). Basal S-CT in these cases was about 2 ng/ml (normal level ≤1 ng/ml) Therefore the question arises whether the determination of basal S-CT alone gives sufficient information for the clinical management of these families. Our experience is in *most* cases yes

It is necessary to use a method for S-CT analysis, which is sufficiently sensitive and specific to discriminate between healthy and tumour-bearing individuals.

Table IV *S-CT response (ng/ml) to calcium infusion in 5 borderline cases*

		(a) First examination			(b) Second examination		
Pat. no.	Age (y)	S-CT at 0	Δ-CT$_{60}$	Δ-CT$_{240}$	S-CT at 0'	Δ-CT$_{60}$	Δ-CT$_{240}$
II:4	28	1 9	0 5	1 4	3 4	2 4	21.6
II:5	22	1 1	0	1 1	0 4	0 4	1.4
III.6	18	0 8	0 1	1 7	1 3	1.8	6 0
III.7	25	1.0	0.3	0 9	0 9	0.3	0 8
III.8	26	0 9	0.3	0.9	0 9	0 9	2 5

Acta

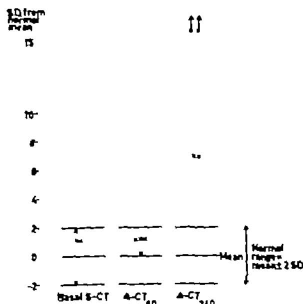


Fig 5 Basal S-CT Δ-CT$_{60}$ and Δ-CT$_{240}$ *in 10 calcium infusion tests in 5 borderline cases of suspected* MCT

Up to now we have *not found any false negative cases of verified MCT* i e all patients have had raised levels of basal S-CT The method for CT determination used by the Boston group seems to be equally sensitive and like ours (1) it allows measurements of normal levels of S-CT (26) Deftos (4) however found elevation of basal S-CT levels in only 26 of 33 patients with MCT i e. the frequency of false negative results was as high as 21% This illustrates the wide overlap of the diagnostic groups and pathological (i e MCT) caused by the sensitivity of the method

However even when a highly sensitive and specific method is used for screening a Sipple family genetically MCT-determined individuals will be found without clinical signs of tumour but with S-CT levels just above the upper normal limit or varying around the upper reference limit. This finding, of course leaves both doctor and patient rather worried. For psychological as well as medical reasons it is desirable to be able to select the early neoplastic cases from this borderline group without having to perform a prophylactic or exploratory total thyroidectomy This operation involves a certain risk of serious complications such as recurrent nerve lesions and hypoparathyroidism Thus additional sensitive diagnostic methods are needed in order to separate the early neoplastic patients from their healthy relatives

It is not known whether the individual patient, genetically determined to develop MCT is born with an abnormal number of C cells or if there is a gradual transition from a normal number through a stage of microscopic 'C cell hyperplasia as beautifully shown by Wolfe et al (31) to macroscopical clinically obvious growth of multiple tumour foci.

As there seems to be a correlation between tumour size and basal S-CT as well as Δ-CT there is probably a similar evolution for S-CT and Δ-CT i e normal–borderline–clearly elevated levels. The concept of a range, which we now call *borderline* regarding Δ-CT$_{240}$ is a definite step forward compared to the preradioimmunoassay era, when only palpable tumours could be diagnosed (see below for further discussion of the staging of MCT tumours)

As to the next goal i.e. the distinction between normal–borderline there must by definition be a statistical limit say between 2 and 3 S D where the high normal and the slightly pathological merge and are difficult or impossible to differentiate except as statistical probabilities Therefore *it is not certain that an increased sensitivity of the determination of S-CT could help us to make this differentiation.*

Δ-CT$_{240}$ in borderline cases

Applying our new test, Δ-CT$_{240}$ (which still utilizes the same laboratory techniques as well as a subtraction with the basal S-CT) might help if there is a difference between basal S-CT and Δ-CT levels in normal and neoplastic tumour cells. Although we do not know why this difference seems to exist. Ten determinations of Δ-CT$_{240}$ in 5 patients gave markedly higher deviations from normal compared with basal S-CT both in absolute concentrations and expressed as S.D from normal mean (Fig 5). Therefore we regard Δ-CT$_{240}$ during induced hypercalcemia as a distinct diagnostic improvement to be applied to the most difficult patient group—the youngest patients harbouring the earliest stages of MCT

Determination of S-CT in sporadic MCT

Also in the non-hereditary MCT the determination of basal S-CT is of great practical value as a diagnostic verification of this tumour In our experience the primary or metastatic tumour in these cases is usually detected as a nodular goiter or an extrathyroidal neck mass It then represents a cell mass large enough to give definitely pathological levels of basal S-CT Thus, determination of Δ-CT$_{240}$ during calcium infusion is of no additional value

Long-term management of MCT patients

Especially in young patients operated upon for MCT it is important to reveal and treat metastatic growth as early as possible. It is well known that this neoplasm has a propensity to metastasize early to regional lymph nodes and then to lungs and bones (6, 7, 11, 13, 16, 30). As yet no effective treatment other than radical surgery can be offered. Therefore we must intensify our diagnostic efforts not only to disclose the primary tumours at an intrathyreoidal stage, but also to detect early recurrencies before spread *outside* regional lymph nodes has occurred. Regular postoperative determinations of Δ-CT_{max} during calcium infusion may give the same additional information as in the very early borderline cases. However, it may be very difficult to localize small lymph node metastases in the fibrotic postoperative tissues without sacrificing parathyroid glands and/or recurrent nerves. Thus different supplementary methods should be tried in order to localize the metastases before explorative surgery. Such a method may be selective venous catheterization with regional sampling for S-CT analysis (10, 28) or selective arteriography of the thyroid, neck and internal mammary arteries, the last of which also supply mediastinal lymph nodes (29, 32).

Sensitivity and specificity of calcium infusion tests

Up to now we have not seen a single case of verified MCT without a significantly pathological rise of S-CT as a C cell response to induced hypercalcemia, i.e. no false negative results have been registered. *False negative* must be defined in relation to the presumed clinical stage of the patient in the evolution of Sipple MCT: 1) Non-palpable tumour and not even C cell hyperplasia. 2) Non-palpable tumour but C cell hyperplasia. 3) Non-palpable tumours of pinpoint to ricegrain size. 4) Palpable tumour (to be further subclassified according to the TMN system).

According to Melvin et al. (17) and Deftos (4) MCT patients may be negative in basal S-CT but positive in Δ-CT. We, too, find that Δ-CT is more sensitive than basal S-CT in group 2 and often in groups 3 and 4, although the suspected tumours of our borderline patients remain to be verified by histopathology.

However, according to the literature the diagnostic results of calcium infusion tests have not been entirely uniform. Three published series of MCT patients (5, 8, 26) showed varying frequencies for the occurrence of false negatives or non-responders to induced hypercalcemia, 0/13, 3/3 and 3/13 patients to be compared with our material (0/14 patients).

There are several explanations of these discrepancies, e.g. (*a*) different sensitivity of the radioimmunoassay techniques, (*b*) different immunologic subspecificity of the antiserum used. Though still directed against human CT, the antiserum may react with different antigenic determinants. (Note different reference values even for assays which are standardized against the same synthetic human CT M (Ciba).)

Furthermore, the evaluation of the published results is difficult as (*a*) The concept of Δ-CT has not been clearly defined and standardized before. (*b*) It seems that calcium infusion tests have not been used systematically on all available MCT patients and Sipple relatives, so that at least some of the published results are in effect selected. This is also due to (*c*) The clinical staging of the patient material is sometimes not presented at all.

As to the response of *normal C cells* to hypercalcemia, it may probably be zero as in some normal controls it seems to be unmeasurable by all published S-CT assays (4, 23, 26). A low assay sensitivity per se will result in inability to measure S-CT levels in many normal subjects even after calcium infusion (4).

As yet we have seen *no* case of verified *false positive* Δ-CT_{max}. False positive may be defined as to two different situations: (*a*) *No tumour at all*. Our control patients with long-standing hypocalcemia might have been expected to give false positive elevations of Δ-CT_{max}, but both had normal responses to hypercalcemia. (*b*) *Non-MCT tumour with endocrine activity*. Either CT proper (ectopic production of CT with biological activity) or only cross reaction in the radioimmunoassay system.

Tumours capable of ectopic production of immunoreactive CT are notably oat cell carcinomas of the bronchus, carcinoids and other tumours of neuroectodermal origin (19, 22). In cases such as these, the diagnosis of MCT should be verified or rejected. We might then possibly make use of the calcium infusion test. The determination of an increased Δ-CT during induced hypercalcemia may then be interpreted as a *functional verification of an abnormal mass of biologically active C cells, i.e. MCT*.

It seems to be a possible hypothesis that non-MCT tumour cells with ectopic CT production would react to hypercalcemia in an atypical way as compared with normal C cells and MCT cells. To the best of our knowledge no such examination has yet been published

From a theoretical point of view the time course of the S-CT rise is interesting with a suggested three-phased course (Fig. 3) It is similar to that of serum insulin after a glucose load. It is reasonable to believe that the secretion processes of the two protein hormones are similar when initiated by a physiological stimulus i e. hyperglycemia or hypercalcemia.

CONCLUSIONS

1 We have introduced and standardized the concept of Δ-CT_{240} as the S-CT response to induced hypercalcemia

2. Δ-CT_{240} is even more sensitive than basal S-CT for the diagnosis of early occult MCT

3 Δ-CT_{240} seems to be normal in patients with chronic hypocalcemia.

4 We recommend the calcium infusion test for (a) screening for MCT in all Sipple relatives with normal or only slightly elevated basal S-CT (b) postoperative control in both sporadic and hereditary MCT (c) investigation of supposed non-MCT tumours with calcitonin production.

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SELECTIVE CORONARY ARTERIOGRAPHY

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Abstract. A series of 414 selective coronary arteriographies in 322 patients has been done at Ullevål Hospital from May 1971 to Jan. 1974. Judkins' technique was used in all cases. The indications for arteriography, the diagnoses as regards the coronary arteries, the therapeutic consequences, and the complications of selective coronary arteriography are described.

Selective coronary arteriography was introduced at Ullevål Hospital in May 1971 with the adoption of open heart surgery. This paper presents the results of a retrospective study of 414 selective angiographies of the coronary vessels in 322 patients examined from May 1971 to the end of Jan. 1974. The review was made in order to help improve the future selection of patients for coronary arteriography in view of the diagnostic yield and the complications with which the method is associated.

METHOD

Judkins' technique for selective coronary arteriography with the transfemoral approach was used in all patients. The patients were examined after a light breakfast with 0.5 mg atropine subcutaneously as premedication. The patients were not heparinized but 5 000 U heparin were added to each 1 000 ml of saline used for flushing the catheters. Left ventriculography was normally done first, followed by selective study of the left and then of the right coronary arteries. Continuous ECG and pressure recordings were done throughout the examination. The contrast media were Urografin 60 % for selective arteriography and Isopaque® Coronar for ventriculography. After completion of the study the femoral artery was digitally compressed for 15 min. A sand bag was thereafter applied and left in place for several hours and the patient was instructed to stay in bed for the rest of the day. Serial ECG and serum transaminase examinations were done whenever the patient complained of severe or long-lasting pain in connection with the procedure or when serious arrhythmias occurred.

MATERIAL

Altogether 414 examinations were done in 322 patients (Table I). The vessels examined are indicated in Table II. In many cases selective examination of the left and right coronary arteries was done in connection with shunt angiography but this has not been included in the material. Duplicate examinations were done as indicated, for technical reasons mostly when the first examination was incomplete or inconclusive. In the Tables to follow correction for duplicate examinations has been made wherever appropriate.

The indications for coronary arteriography are shown in Table III. Coronary heart disease in total, i.e. the first six indications in Table III put together, accounted for 78 % of the indications for coronary arteriography (men 84 %, women 61 %). Valvular heart disease accounted for the remaining 22 % of the indications (men 16 %, women 39 %).

RESULTS

Diagnosis

The distribution of coronary artery disease in this study is shown in Table IV. The group "normal" comprises most of the patients with valvular disease as well as some with angina pectoris. Six of the eight patients with "shunt occluded" also had at least one shunt patent. Vice versa 6 of the 67 patients with "shunt patent" also had at least one shunt occluded. By "single vessel disease" is meant at least 50 % stenosis of either left anterior descending, circumflex, or right coronary artery or a major branch of them. Correspondingly "double" and "triple vessel disease" refer to similar affection of two or three of these arteries. Table IV gives the relative distribution of single, double and triple vessel disease. This distribution was similar for men and women.

Of the 103 patients with normal findings or only minor changes in the coronary arteries the indication for arteriography was valvular disease in 53 cases, i.e. almost exclusively aortic valvular disease, as coronary arteriography was not often done in patients with mitral disease. In the remaining 50 cases the indication was coronary artery disease in one manifestation or another or query coronary disease.

Table I *Total material*

	No of examinations	No. of pats.	Age (y) Range	Mean
Men	332	250	18–80	53
Women	82	72	25–70	57
Total	414	322		

Table II *Vessels examined*
LCA = left coronary artery RCA = right coronary artery

	Total no of examinations	Duplicate examinations
LCA + RCA	323	12
LCA	10	2
RCA	5	2
Early shunt	60	–
Late shunt	16	1
Total	414	17

Of the latter group many patients ultimately got the diagnosis of cardiomyopathy Four patients with normal coronary arteries had circumstantial evidence of coronary artery disease such as previous myocardial infarction or positive ECG findings in addition to angina pectoris

Among 55 patients studied for valvular disease alone there were 7 men and 2 women who had significant coronary artery disease (50% stenosis or more) One man was only 25 years old He had aortic stenosis and insufficiency and a hypoplastic right coronary artery with significant atheromatous changes He had a negative family history and no serum lipid disturbances. Another man in this group was 52 years old. The remaining 7 patients were all over 60 years of age

A total of 97 shunts in 69 patients were studied by selective angiography Eighty-one shunts were studied at an early stage (early shunts) i.e before the patients left hospital after bypass surgery Of these 75 shunts were found to be patent and 6 were occluded One occluded and 10 patent shunts were in studied at a later stage 2–17 months postoperatively The one occluded shunt remained occluded and there were no further occlusions among the patent shunts. Another 16 shunts were studied at a late stage only (late shunts ") 3–17 months postoperatively Twelve of these were found to be patent and 4 were occluded Altogether there were 10 occluded shunts i the material Flow was measured peroperatively in 7 of them. The flow values ranged from 20 to 50 ml/min (mean 39) Flow was measured in 84 of the 87 shunts which remained patent It ranged from 10 to 180 ml/min (mean 77)

Therapeutic consequences

Of the 322 patients examined 125 were operated on (Table V) The remaining patients were not operated on for reasons given in Table VI

Most of those with normal findings were patients examined for query coronary heart disease who turned out to have normal coronary arteries Of the patients who died before operation, 3 died in relation to coronary arteriography and 6 while await

Table III *Indications for coronary arteriography*

In some cases there was more than one indication for arteriography figures within parentheses represent cases with only one such indication

	No. of examinations Men	Women	Total	% of total
Angina pectoris	149 (129)	34 (26)	183	41.3
CHDT*	39 (38)	12 (11)	51	11.5
Unstable angina / Imminent infarction	14 (14)	2 (2)	16	3 6
Arrhythmias	11 (8)	1 (1)	12	2.7
Cardiogenic shock	2 (2)	–	2	0.5
Aneurysm	23 (17)	1 (1)	24	5 4
Valvular disease	47 (37)	32 (23)	79	17.8
Postoperative shunt angio	69 (69)	7 (7)	76	17.2

* Query coronary heart disease.

Table IV *Distribution of coronary artery disease*

Figures within parentheses indicate the relative distribution

Diagnosis	No. of diagnoses	Distribution (%)
Single vessel disease	54	13 (26)
Double vessel disease	80	20 (37)
Triple vessel disease	79	20 (37)
"Normal" (<50 % stenosis)	103	26
Shunt patent	67	16
Shunt occluded	8	2
Incomplete study	11	3

ing surgery Three patients were randomized to medical treatment in a special study and therefore not operated on.

Seventeen patients underwent coronary arteriography as an emergency procedure in order to have immediate surgical treatment if possible The indications for arteriography the complications of arteriography and the subsequent therapeutic consequences as regards surgery as well as the ultimate fate of these patients are given in Table VII

Complications

The serious complications of coronary arteriography in this material as registered in the case records are shown in Table VIII The distinction between manifest and "possible myocardial infarction (AMI) rests on the presence or absence of unequivocal pathological changes in the ECG or serum transaminases, in addition to the clinical picture of AMI The group serious arrhythmias" included one case of complete AV block, two cases of ventricular tachycardia, and four cases of ventricular fibrillation (VF) In addition VF occurred in 4 of the 11 patients with manifest or possible AMI.

Details of the three deaths

Case 1

A 70-year-old woman with several syncopal attacks over the past few years She was admitted to hospital after another syncope. On admission she had atrial fibrillation which later reverted to sinus rhythm. She had aortic stenosis and mitral insufficiency and underwent left ventriculography and coronary arteriography in assessment for operative treatment. The study was completed without complications. Several hours later the patient was found to have large hematoma in the groin By then the sand bag had been removed She developed shock and VF and died in spite of attempted resuscitation. At autopsy there were no signs of AMI

Table V *Patients operated on*

Operation	Men	Women	Total	%
Aorto-coronary bypass	61	11	72	58
Valvular repair	19	10	29	23
Aneurysmectomy	9	1	10	8
Bypass + valvular repair	4	2	6	5
Bypass + aneurysmectomy	7	1	8	6
Total	100	25	125	100

Case 2

A 50-year-old man with mitral insufficiency and angina pectoris. On admission he had severe left ventricular failure but improved with treatment. During left ventriculography and coronary arteriography he became distressed with signs of pulmonary congestion and cerebral embolism. The study was interrupted The patient died 3 days later Autopsy showed a large hemorrhagic infarction in the left hemisphere.

Case 3

A 69-year-old woman with aortic and mitral valvular disease. On admission she had acute pulmonary edema. Following improvement she underwent angiography which also revealed central stenosis of the left coronary artery The angiography had to be interrupted because the patient developed hypotension and pulmonary edema. She died 12 hours later Autopsy showed evidence of AMI.

Of the 21 patients who developed manifest myocardial infarction (7) possible myocardial infarction (4) or serious arrhythmias (7) or died in relation to coronary arteriography (3) 20 were completely studied Four of them had normal coronary arteries three had single vessel disease four double vessel disease and nine triple vessel disease

Table VI. *Patients not operated on*

In some cases there was more than one reason for not operating: figures within parentheses represent cases with only one such reason

Reason	Men	Women	Total	%
Technically inoperable	53 (50)	8 (8)	61	31.0
Poor risk	15 (12)	7 (6)	22	11.2
Patient refused	5		5	2.6
Normal findings	35	19	54	27.4
Awaiting operation	9	7	16	8.1
Symptoms too mild	25 (21)	2 (1)	27	13.7
Died before operation	4	5	9	4.5
Randomized	3		3	1.5

DISCUSSION

The present material showed a marked dominance of men to women among patients submitted to selective coronary arteriography It also appeared that the men were somewhat younger than the women in this group of patients The male dominance was even more pronounced among patients examined for coronary heart disease alone whereas there was a more even sex distribution among patients examined for valvular disease (Tables I and III) As expected angina pectoris was the single most common indication for coronary arteriography but among women it was nearly equalled by valvular disease

Of the patients with a definite angiographic diagnosis of significant coronary artery disease only one fourth had single vessel disease The remainder had more widespread coronary artery disease making them strong candidates for aorto-coronary bypass surgery

The number of normal findings as regards the coronary arteries among the total number of diagnoses amounted to 25 % (103 of 409) This may seem a high figure but only half of these (50 patients) were studied for coronary heart disease In the remaining 53 cases with normal coronary arteries the indication for arteriography was valvular disease The question arises whether or not it was justifiable to submit the patients with valvular disease to the potential hazards of selective coronary arteriography in the absence of angina pectoris In aortic valvular disease the indication for selective coronary arteriography is twofold Firstly there is need for exact knowledge of the anatomical relationship of the coronary arteries to the valve region secondly there is the need to diagnose coexisting coronary artery disease The known coexistence of aortic valvular and coronary disease due to the common underlying disease of athero-

Table VII *Patients studied as emergency cases*

Indication	No of pats	Complications AMI	Complications VF	Operated on	Alive
Imminent infarction	10	2		7	9
Arrhythmias	5		1	4	4
Cardiogenic shock	2	–		1	2
Total	17	2	1	12	15

Table VIII *Complications of coronary arteriography*

	Men	Women	Total	% of total material
Deaths	1	2	3	0 7
Manifest AMI	5	2	7	1 7
Possible AMI	4	–	4	1 0
Cerebrovascular accident	2	–	2	0.5
Local hematoma or bleeding	2	–	2	0.5
Serious arrhythmias	6	1	7	1 7
Femoral artery aneurysm	1	–	1	0.2

sclerosis was verified in the present material by the fact that of the 55 patients who underwent arteriography for valvular disease alone i e without having angina pectoris 9 patients (16 %) were found to have significant coronary artery disease Most of these patients were men and most of them were elderly

In this material of 322 patients 4 had normal coronary angiograms in the presence of what was regarded as definite coronary heart disease A small number of such patients must be accepted in this kind of material Whether it may be explained by occlusion of an arterial branch flush with the main vessel by postulated small vessel disease or by faulty technique of study remains uncertain

The relationship between shunt occlusion and low shunt flow as measured intraoperatively is well known (5) This relationship was verified in the present material by the observation that the shunts that occluded had on an average lower flow values (mean 39 ml/min) than the shunts that remained patent (mean 77 ml/min) But there was considerable variation and some of the shunts that remained patent had intraoperative flow values as low as 10–20 ml/min even after stimulation with temporary occlusion or intravascular papaverine It is perhaps worth noticing that one of the two Y-grafts in the series occluded In the few cases in whom repeat shunt angiographies were done no further occlusions were found on the second examination indicating that graft occlusion occurs early (2 6)

Most of the 125 patients who came to operation had aorto-coronary vein bypass or valvular repair (Table V) The remaining 197 patients were not operated on for reasons given in Table VI The

commonest reason for not operating was technical inoperability which accounted for 61 cases (31 %) or nearly 20 % of the total patient material This figure may vary from centre to centre according to the local criteria for inoperability

Two of the reasons for not operating require special attention One is the situation where the patient refuses operation Ideally no patient should undergo potentially dangerous investigations if he has already decided not to submit himself to the treatment at which the investigation is aimed The second reason is the situation where the patient s symptoms are too mild to warrant the risk of surgery In 22 cases this was the only reason for not operating in the present series Clearly these patients should not have been submitted to coronary arteriography in the first place

In a retrospective study like the present the incidence of complications of selective coronary arteriography may well be underestimated. However there is no doubt that the figures in Table VIII truly represent the actual incidence of complications as regards deaths, manifest and possible infarction cerebrovascular accidents whereas the true figures for hematomas and arrhythmias are probably higher than those given in the Table.

Takaro et al. (8) found that more than half of the 66 deaths occurring in 3 044 coronary artery investigations were caused by acute coronary occlusion, mainly by catheter emboli. In one of our patients died from AMI there was no evidence of ...olus and the most likely mechanism was thought to be coronary artery occlusion by wedging catheter tip in the coronary ostium In another our patients however who died from a massive cerebral infarction a stripped-off catheter embolus have been the cause of death. In the patient who died from a local groin hematoma, death must partly be blamed on insufficient patient monitoring and this was therefore the most preventable death in the series

Considering that all three patients who died in our series were critically ill a mortality rate of 0 7 % may acceptable in view of the important information yielded by the study

Several authors have pointed out recently that there seems to be a marked difference in the frequency of serious complications of selective coronary arteriography between the femoral (Judkins) the brachial (Sones) techniques In papers two techniques mortality rates with the femoral approach have been reported to be 2 4 % (7) 1 4 % (9) and 2.2 % (6) in each case considerably higher than the mortality rate with the brachial method Adams et al (1) for instance found that the mortality related to the femoral technique was 6 times that of the brachial technique in a large nationwide survey from 173 hospitals in the US Other serious complications were also much more common with the femoral technique. The greater tendency for complications to occur with the femoral technique has been explained partly by the increased risk of complete coronary artery occlusion by the preshaped Judkins catheters (7 9) and partly by the need to change catheters and by the use of guide wires which in themselves have definite thrombogenic properties (4 8) Petch et al (7) also thought that there was a greater tendency for the Judkins catheters to stimulate the aortic baroreceptors and thereby cause reflex bradycardia and hypotension which could be fatal. On the other hand it has been claimed that the femoral technique tends to be adopted by the less skilled investigators since the coronary ostia are more readily entered by the preshaped Judkins catheters The greater complication rate with the femoral technique seen in some centres may therefore to large extent depend on lack of experience and technical skill (3). Not only will faulty technique in itself provoke a high incidence of complications, but undue prolongation of the study may further increase the risk of thromboembolic complications With increasing experience the rate of complications with the femoral technique is drastically reduced. Thus Adams et al (1) found that the mortality was reduced by a factor of 8 in centres which did more than 800 studies per 2 years as compared to centres which did less than 200 studies per 2 years It is our conclusion, therefore that centres which are already familiar with the femoral technique and have gained some experience in dealing with the potential hazards of coronary arteriography as we feel is the case in our hospital need not abandon the femoral technique i order to reduce the rate of complications. A change to the brachial technique may well increase rather than decrease the number of complications at least temporarily and the number of unsuccessful studies would certainly increase

It has been pointed out that the bolus of heparin given routinely to the patients with the brachial technique may serve to reduce the number of thromboembolic complications Judkins

(3) therefore now advocate the use of a single dose of heparin also with the femoral technique. So far this has not been the routine in our hospital.

Three of the seven patients with manifest myocardial infarction and one of the four with possible myocardial infarction associated with the arteriography in this series were successfully operated on with aorto-coronary bypass at a later stage. Therefore, patients are not necessarily worse off in the end even after having sustained an infarction during selective coronary arteriography than they would have been if left uninvestigated.

One would expect a high complication rate in patients studied as emergency cases in assessment for immediate surgery. In fact such emergency arteriographies accounted for only two of the cases of definite myocardial infarction. The rest of the complications, including the deaths, occurred in patients studied electively. As shown in Table VII, 12 of the 17 patients studied as emergency cases were operated on, and 15 of the patients have survived to the present date. In properly selected patients it may therefore be well worthwhile carrying out emergency coronary arteriography, and the rate of complications does not seem to be unduly high.

Takaro et al. (8) pointed out that the great majority of patients who died in connection with selective coronary arteriography had triple vessel disease. The present study confirms the high frequency of widespread disease among patients with serious complications, but it also appeared that some of these patients had single vessel disease only or even normal coronary arteries.

Selective coronary arteriography is associated with a definite incidence of serious complications as shown by this and other studies. It is therefore evident that candidates for coronary arteriography must be carefully selected. The patients who will refuse operation and those with symptoms too mild to warrant operation should not be submitted to coronary arteriography, as pointed out earlier. One debatable question is whether or not it is justifiable to submit patients to routine shunt angiography following aorto-coronary bypass. In the present series there were 76 shunt studies, most of which included repeat selective coronary arteriography as well.

The total complications of shunt angiography were one possible myocardial infarction and one local groin hematoma. With this very low incidence of complications it is presently felt that routine shunt studies should be done in order to evaluate the results of the bypass operation. When considering the risks of selective coronary arteriography, it is appropriate to note that in the present material two patients with valvular disease and four with coronary disease scheduled for surgery died before their date of operation. This amounts to 2% of the total material, which is higher than the combined mortality for selective coronary arteriography and aorto-coronary bypass operation in this hospital.

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AORTOCORONARY VEIN BYPASS IN PATIENTS WITH ANGINA PECTORIS

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Abstract In the 3-year period from May 1971 to April 1974 90 patients had aortocoronary bypass for angina pectoris at Ullevål Hospital. One patient died shortly after the operation (operative mortality 1 1%). There were no further deaths in the observation period Clinical improvement was seen in 93% of the patients, early shunt patency in 92%. The study suggests that patients with isolated affection of the right coronary artery should not have bypass, because these patients 1) had less severe symptoms, 2) had better preserved left ventricular function, and 3) seemed to have a smaller chance of benefiting from the operation than the other patients. Multiple shunts gave good clinical results and carried no higher surgical risk than did single shunts. Good clinical results were seen also in patients with occluded shunts provided they had at least one patent shunt too. Graft occlusion occurred early and was associated with low graft flow as measured intraoperatively Graft occlusion was not usually followed by demonstrable myocardial necrosis. In view of the small operative risk and the high score of symptom relief it is concluded that all patients with angina pectoris that does not readily respond to medical treatment, should be considered for aortocoronary bypass.

Since its introduction in 1967 (6) aortocoronary bypass has gained wide acceptance in the treatment of ischemic heart disease This is largely due to the immediate and often dramatic relief of angina pectoris following the bypass operation Thus improvement in the quality of life has been and still is the aim of this treatment.

The final position of the bypass operation in the management of coronary heart disease is however not yet defined. It will depend on long-term postoperative survival and infarction rates information about which is not available The effect on short term prognosis, i.e. operative and postoperative mortality and morbidity is currently subject to much discussion the outcome of which must also influence the future position of this treatment.

The purpose of this paper is to present a material of patients treated with aortocoronary vein bypass for angina pectoris which we think will demonstrate that the operative risk can be very small and the clinical results very good The purpose is also to discuss some aspects of coronary artery disease in this material in relation to symptoms and results.

MATERIAL

The material consists of 90 patients, 77 men aged 28–65 years (mean 51) and 13 women aged 25–65 years (mean 51), who had aortocoronary vein bypass for angina pectoris during the 3-year period from May 1971 to April 1974 at Ullevål Hospital. The follow-up time was 2–37 months (mean 14). None of the patients had additional heart surgery During the same period a number of other patients had bypass surgery plus valve repair or aneurysmectomy These patients will be presented in separate study and are not included in the present paper

In 67 of the 90 patients the indication for bypass surgery was stable angina pectoris which medical treatment had failed to relieve satisfactorily Twenty patients had unstable angina or imminent infarction. By unstable angina was understood the debut or marked aggravation of chest pain in the period prior to the operation. By imminent infarction was understood recurrent or long-lasting pain in hospital and/or transient ischemic changes in the ECG in patients admitted to hospital with acute coronary symptoms in whom the diagnosis of established acute myocardial infarction (AMI) was excluded. One patient was operated on for AMI. In two patients the indication for operation was coronary artery disease and serious arrhythmias resistant to medical therapy

Patients above 65 years of age and patients with manifest left ventricular (LV) failure in spite of medical treatment were not operated on. AMI more than few hours but less than 3 months old was another contraindication to operation. Hypertension abnormal serum lipids, and previous myocardial infarction older than 3 months were not regarded as contraindications to surgery

The vessels affected by significant coronary artery dis-

Table I *Vessels affected*

LAD=left anterior descending artery CF=circumflex artery RCA=right coronary artery

Vessels	No of pats.		No of vessels
LAD	8	16	Single vessel disease
CF	0		
RCA	8		
LAD+CF	11	37	Double vessel disease
LAD+RCA	20		
CF+RCA	6		
LAD+CF+RCA	37	37	Triple vessel disease

ease, i.e 50% stenosis of the lumen or more are shown in Table I Most patients had double or triple vessel disease The left anterior descending (LAD) artery was affected in 76 patients, the right coronary artery (RCA) in 71 patients, and the circumflex (CF) artery in 54 patients. Thus, affection of the CF artery was a little less common than affection of the other two main arteries in this material of operated patients.

In the 90 patients there was total of 135 grafts divided into 50 single 35 double, and 5 triple grafts. Multiple grafts were separate in all but two cases, in whom a Y graft was used The majority of the grafts were anastomosed to the LAD artery and to the RCA End arterectomy was not done routinely but in 14 coronary arteries it proved necessary in order to obtain good distal graft to artery anastomosis or good distal run-off

METHOD

All patients had bicycle exercise test, right heart catheterization left ventriculography with recording of pressures before and after contrast and selective coronary angiography by Judkins technique preoperatively The operation was done in moderate hypothermia with temporary clamping of the aorta and temporary cardiac standstill The great saphenous vein was used for grafting. Coronary vessels with internal diameters down to 1 mm were accepted for distal anastomosis Intraoperative graft flow was measured with an electromagnetic flowmeter Anticoagulant therapy was started postoperatively and continued for about 3 months after the operation.

In most patients selective graft angiography was done 2 3 weeks after the operation, i.e before the patients were discharged

Follow-up angiographic and hemodynamic studies, several months postoperatively were only done in some patients, mostly those with residual symptoms. Late graft angiography and hemodynamics were therefore only obtained in these selected cases

RESULTS

Complications

Of the 90 patients operated on, one has died (overall mortality 1 1%) He was a 48-year-old man with chest pain at rest thought to have an imminent infarction He died 5 hours after the operation. Autopsy showed that he had in fact an AMI about 48 hours old and he should therefore not have been operated on according to the criteria above

The other complications are listed in Table II There were 4 AMIs in connection with the operation (4 4%) and another 2 patients with the clinical picture of AMI but no definite ECG changes Most of the AMI patients nevertheless showed clinical improvement following surgery (vide infra). All patients with postcardiotomy syndrome, wound infection and pericardial bleeding made a good recovery There were no further deaths in the follow-up period but one patient had a small myocardial infarction 14 months after the operation and another patient had a possible infarction in the follow-up period

A possible relationship between the number of shunts in each patient and the peroperative complications was looked into In the 5 patients with triple shunts there were no serious complications In the 35 patients with double shunts there were three manifest infarctions and in the 50 patients with single shunts there were one death due to myocardial infarction, one other manifest and two possible myocardial infarctions. Thus, there was no increase in complications in the patients who had multiple shunts

Clinical results

The clinical results are based on the clinical assessment of the severity of angina pectoris before and after bypass surgery Seventy-three patients

Table II *Complications*

Follow-up time 2 37 months (mean 14)

Complication	No of pats.	% of total pat. material
Peroperative		
Death	1	1 1
Manifest infarction	4	4 4
Possible infarction	2	2.2
Postcardiotomy syndrome	3 (+4?)	3 3
Wound infection	3	3.3
Pericardial bleeding	3	3.3
After discharge		
Death	0	
Manifest infarction	1	1 1
Possible infarction	1	1 1

Table III. *Correlation between complications and clinical results*

	Clinical results (no. of pats.)			
Complication	Much impr	Im-proved	No change	Worse
Peroperative				
Death				1
Manifest infarction	3		1	
Possible infarction	2			
Postcardiotomy syn-drome	7			
Pericardial bleeding	2	1		
After discharge				
Manifest infarction		1		
Possible infarction		1		

(81 1%) were greatly improved and 10 patients (11 1%) were distinctly improved after the operation. Six patients (6 7%) did not benefit from surgery their symptoms were unchanged but not worse. One patient (1 1%) died. Thus 93% of the patients were either much or distinctly improved ...er surgery

Of 76 patients with a postoperative follow-up time of more than 3 months 63 patients (83%) returned to work. Five patients (7%) did not go back to work because of persistent angina pectoris Another 8 patients (10%) did not return to work for ... other than cardiac. They were all clinically ...mproved after the operation.

The clinical results were good even in patients ... peroperative and late complications Table III ... the relationship between complications and ...linical results

The patient who was operated on for AMI was a ...-year-old man who arrived in hospital one hour ... the onset of pain. ECG showed an extensive ...erior infarction. A vein graft was put to his ...nterior descending artery 5 hours after admission. ...he immediate effect was a distinct reduction in the ... of ischemia surrounding the infarcted area. ...he patient made an uneventful recovery

...unt patency

...the 135 shunts in the material 91 were examined ...y *selective shunt angiography shortly after the op*...ration (Fig. 1). Eighty-four shunts (92%) were patent and 7 (8%) were occluded. Thirty-one shunts were examined at a late stage on an average 12 months postoperatively Twenty-six shunts (84%) were patent and 5 (16%) were occluded. The total number of occluded shunts at early and late examination was 11 and the total number of patent shunts was 97 Mean flow in the patent shunts measured during the operation was 74 ml/min and in the occluded shunts 39 ml/min One patient with a single shunt and another patient with two shunts had all shunts occluded. All the other patients had at least one shunt patent. Fourteen shunts were examined both early and late postoperatively One was occluded on both occasions and 13 were patent on both occasions Thus occlusion occurred early and there were no further occlusions between early and late angiography in these shunts

Shunt occlusion was not usually followed by demonstrable myocardial infarction. On the contrary most infarctions occurred in patients with patent shunts Thus, 3 of the 5 manifest and 2 of the 3 possible infarctions occurred in patients with all shunts patent. One manifest and one possible infarction occurred in two patients with one or more

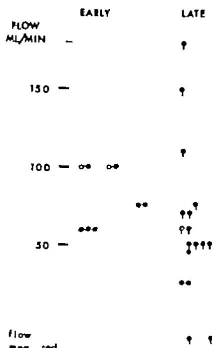


Fig. 1. Shunt patency related to intraoperative shunt flow in 91 shunts examined early (1–... weeks), and 31 examined late (average 12 months) postoperatively O=patent shunts, ●=occluded shunts, vertical line=shunt also examined early

Table IV *Correlation between vessels affected and LV function*

PCV = pulmonary capillary venous pressure (during exercise) LVEDP = left ventricular end-diastolic pressure (after ventriculography) N patients had isolated CF affection

Vessels affected	Mean PCV (mmHg)	No of obs.	Mean LVEDP (mmHg)	No of obs
Single vessel disease				
LAD	22 4	5	18.5	6
RCA	16 8	6	14.8	5
Double vessel disease				
LAD+CF	[illegible]0	7	19 7	10
LAD+RCA	[illegible]4 7	11	[illegible]0 1	16
CF+RCA	[illegible]4 1	6	21 4	5
Triple vessel disease				
LAD+CF+RCA	[illegible]4 7	24	22.0	29

shunts occluded. In one patient with manifest infarction postoperative shunt angiography was not done

Hemodynamic results

Most patients had preoperative assessment of LV function by registration of pulmonary capillary venous pressure (PCV) during supine bicycle exercise and LV end-diastolic pressure (LVEDP) following left ventriculography Table IV shows correlation between these parameters and extension and localization of significant coronary artery disease PCV and LVEDP were elevated in all groups of patients except those with isolated affection of the RCA

There was a limited number of comparable observation as regards LV function before and after the operation Fig shows the effect of the operation on LVEDP following left ventriculography in 8 patients and on LV ejection fraction (LVEF) in 1 patients LVEF was estimated from the left ventriculogram using the single plane area length method of Sandler and Dodge (13) LVEDP was reduced in 7 of 8 patients after the operation A reduction was also seen in one patient with a single shunt that was found to be occluded LVEF was increased in 9 of 12 patients after the operation, all 9 had patent shunts Two of the three patients who showed a reduction in LVEF postoperatively had patent shunts while the third patient had a single shunt that was occluded Of 15 patients with pre and postoperative left ventriculograms 7 had LV enlargement preoperatively Only two of these showed a reduction in LV size postoperatively In the remaining 5 patients, all of whom had patent shunts the LV enlargement persisted One patient with a normal preoperative ventriculogram had an enlarged left ventricle postoperatively He had a single shunt that remained patent, but he had a possible myocardial infarction in connection with the operation. In only 3 of 6 patients with patent shunts who had preoperative LV dyskinesia was LV contraction seen to be normal postoperatively Shunt patency was therefore poorly related to improvement in LV dysfunction as expressed by LV enlargement or dyskinesia.

DISCUSSION

Indications for surgery

It is widely accepted that patients with stable disabling angina pectoris, resistant to medical treatment should be offered surgery Most of the patients in our material fell into this category It is our belief however in view of the good clinical results and the low mortality associated with the operation, that bypass surgery should be offered also to patients with somewhat less severe angina pectoris, provided the symptoms are severe enough to interfere with their daily activities. For the same reasons we think that patients with unstable angina

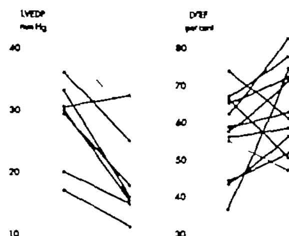


Fig 2 LVEDP immediately following ventriculography in 8 patients before and after bypass surgery LVEF in L patient before and after bypass surgery = one patient with the only shunt occluded. The remainder had patent shunt

and imminent infarction should have the benefit of bypass surgery too. Most of these patients have in fact had previous stable angina which would make them candidates for surgery on the basis of symptom relief. The additional possibility of preventing an AMI should strengthen the indication for operation. In patients with angina pectoris of short duration, without previous symptoms it has been our policy however to delay surgery in the expectation of spontaneous improvement.

AMI is a special indication for aortocoronary bypass. In all reports the number of patients operated on is small, but most authors claim good results if the interval between the onset of symptoms and the operation does not exceed 5–6 hours (3 14) which is considered to be the limit for reversibility of myocardial ischemia. Our AMI patient demonstrated that the operation may be well tolerated and the area of permanent myocardial damage may be reduced.

The bypass operation carries a definite risk of serious complications and the selection of patients therefore has to be strict. A small mortality 1 1% in our material, must, however be accepted with this kind of treatment and weighed against the high score of symptomatic relief 93% in our material It should be noted moreover that while there was one operative death 4 other patients in this hospital scheduled for bypass surgery died suddenly before the date of their operation, even though the waiting time for surgery was less than 6 weeks

When discussing the complications of bypass treatment, one must also take into account the risks of the preoperative selective coronary arteriography and left ventriculography So far there have been no fatal complications to preoperative angiography in patients investigated for coronary disease in this hospital (9) and no patients have been refused subsequent bypass surgery because of complications in relation to preoperative angiography

Even in the presence of operative complications, most patients were improved clinically following surgery (Table III). Thus, 4 of 5 patients with definite myocardial infarction showed clinical improvement, and they all returned to work. This may possibly be explained by the fact that intraoperative infarctions tend to be small and of limited consequences. It also appeared that the postcardiotomy syndrome had no derogative effect on the final clinical result.

Single–double–triple vessel disease

In this material as in others most patients had double or triple vessel disease but 16 of the 90 patients operated on had lesions limited to a single main coronary vessel. Since these patients have a good prognosis of survival without surgery it is debatable whether they should be submitted to the risk of the operation. The operative mortality is so small, however especially in these patients with single vessel disease who are usually good risk patients that it should not be a major contraindication to surgery when the symptoms are severe, and the only efficient way of relief is by means of aortocoronary bypass That patients with single vessel disease may have just as severe symptoms as patients with multiple vessel disease is demonstrated by the fact that in this material as many as 1/3 of the patients with single vessel disease had unstable angina or imminent infarction To some extent this may be a reflection of patient selection because of patients with single vessel disease only those with severe symptoms were operated on. Patient selection was probably also the main explanation why there were no patients with isolated CF artery disease in this material since these patients, because of the technical difficulties associated with bypass to the CF artery would only be operated on in the presence of very severe symptoms.

There seemed to be a difference in severity of symptoms between the 8 patients who had isolated LAD involvement and the 8 who had isolated RCA involvement. In the former group, 4 of the 8 patients had unstable angina or imminent infarction, but in the latter group only one of the 8 had such severe symptoms. It is difficult to see why there should be such a difference unless it reflects a difference in collateral blood supply to the areas supplied by the two arteries.

If one looks at the relationship between arteries affected and clinical results in this material it is seen that in all 37 patients with triple artery involvement there was much improvement (34 pats) or improvement (3 pats.) following the operation. Likewise of the 37 patients with double artery involvement the majority showed much improvement (28 pats) or improvement (6 pats.) after the operation. Only 3 patients with combined LAD and RCA involvement were unchanged after the operation. In patients with single vessel disease however there was again a difference between patients with iso-

lated LAD disease and patients with isolated RCA disease In the former group 7 of 8 patients were much improved and one patient was improved In the latter one of the 8 patients died and another 3 were unchanged after the operation. Thus patients with isolated RCA disease only showed improvement in 50% of cases.

Number of shunts

Of the 40 patients who had double or triple shunts 33 patients were much improved and 6 were improved after the operation. Multiple shunts therefore gave good clinical results. Of the 50 patients with single shunts 6 failed to improve. It was concluded that multiple shunts gave a better chance of symptom relief than did single shunts and the risk of complications was no higher (vide supra)

Shunt patency

The great majority of the shunts studied by angiography postoperatively were patent. Of 55 patients who had all shunts patent at the early post operative study 45 showed much clinical improvement and 6 showed improvement, whereas 4 patients had no change in symptoms after surgery Thus about 7% of the patients did not benefit from the operation in spite of early postoperative shunt patency Of the 79 patients with all shunts patent at late shunt angiography three (10%) failed to show inical improvement. On the other hand, all three ents who had one of two shunts occluded at y shunt angiography nevertheless showed uch clinical improvement, and one patient with two of three shunts closed was also much improved clinically as was one patient in whom the only shunt was occluded at late shunt angiography There was therefore not a good correlation between shunt patency and clinical results. Determination of shunt patency must therefore rely on shunt angiography

The discrepancy between postoperative symptoms and shunt patency is not difficult to explain. Even with all shunts patent there may still be areas of the myocardium that remain underperfused thereby maintaining the symptom of angina pectoris. On the other hand, relief of angina pectoris in patients with occluded shunts is seen when there are other shunts that remain open with a good collateral blood supply The finding of all shunts occluded in the presence of good clinical improvement as in one of our patients is less readily explained and a placebo effect cannot be excluded in such cases.

Shunt occlusion was directly related to shunt flow as measured intraoperatively No shunts with a flow over 50 ml/min occluded This is in agreement with other reports (10) The main factor determining shunt flow is the state of the peripheral vascular bed It is clearly necessary therefore, to have a good distal run-off and patients with poor peripheral vessels should not be operated on. If the stenosis in the coronary vessel to be bypassed is only slight or moderate the coronary vessel may steal too much flow from the bypass graft and thereby cause graft occlusion This was not felt to be the case in any of our patients as all bypassed vessels had at least a 50% reduction in diameter

In our material all shunts that were patent at early shunt angiography remained patent also at late shunt angiography suggesting that shunt occlusion occurred early postoperatively if it occurred at all. Late shunt occlusion sometimes demonstrated to be the result of progressive fibrosis of the vein graft (4) the so-called graft disease was not observed in any of our patients.

Other reports have shown that shunt occlusion occurs in 5–15% of cases (5 11) In our material 16% of the shunts examined at a late stage were occluded It must be pointed out however that late shunt angiography was mainly done in patients with residual symptoms and the occlusion frequency found may therefore be higher than in the material as a whole

Most of the infarctions occurred in patients who had patent shunts This supports the assumption that infarctions that do occur are located in areas not supplied by the coronary vessel operated on. Vice versa in the majority of patients with occluded shunts there were no demonstrable signs of myocardial infarction. It may be that in these patients the area supplied by the shunt was already fibrosed Another possibility is that the area supplied by the shunt had a good collateral supply from other vessels. In each case the shunt would really be surplus and it would carry little blood which in turn would provoke early shunt occlusion. If these explanations for some cases of early shunt occlusion are accepted the problem clearly is one of preoperative assessment. So far our preoperative studies cannot with confidence tell us whether a particular area of the myocardium is

1) fibrotic beyond possibility of improvement through improved blood supply or 2) sufficiently perfused via collaterals from vessels other than the native vessel

Hemodynamics

It is interesting to look at the correlation between arteries affected by significant coronary disease and signs or LV failure preoperatively Since patients operated on were not in manifest LV failure PCV during exercise and LVEDP following left ventriculography were chosen as indices to bring out any signs of latent LV failure Table IV shows that the mean values for PCV and LVEDP for all groups were pathologically elevated with the exception of patients with isolated RCA disease It is concluded therefore that these patients with angina pectoris without clinical heart failure nevertheless had latent LV failure its degree being independent of the extent of the coronary artery lesions as demonstrated by selective coronary arteriography The possible exception to this was patients with isolated RCA disease, who seemed to have better preserved LV function. This is probably explained by the fact that the RCA contributes relatively little to the blood supply of the left ventricle.

Thus, it seems that coronary artery disease limited to the RCA was tolerated well by the patient both with respect to symptoms (vide supra) and with respect to LV function.

The good clinical results in this material are similar to those reported by others (7 11) It is assumed that relief of symptoms is mainly due to increased myocardial perfusion although definite proof of this is difficult to obtain. Attempts have been made to correlate symptom relief to improvement in LV function, which is thought to be a better indication of increased myocardial perfusion. It should be born in mind however that since these patients usually have normal or near normal LV function at rest preoperatively it may be difficult to demonstrate any improvement postoperatively unless the tests for LV function are made under some kind of stress. Some reports in the literature demonstrate no change in LV function following aortocoronary bypass although symptomatic relief from angina is good (1 8). Others claim *improvement in LV function* too (2, 12) Our observations did suggest some degree of improvement in LV function as expressed by LVEDP after left ventriculography and by LVEF In most of the patients who had comparable pre- and postoperative studies but the observations were too few to allow definite conclusions.

Limitations of the study

There are two main limitations in a study like the present Firstly there is the subjectiveness on which the clinical results are based Considering that the symptom of angina pectoris is quite readily influenced by a number of factors, many of which must undoubtedly have a placebo effect it is an obvious drawback that the evaluation of the results should rest on the patients experience of chest pain In our opinion however having seen the patients before and after the bypass operation, it is quite clear that no placebo effect could be wholly responsible for the striking and uniform improvement in the clinical condition of most of these patients the great majority of whom were able to return to full time work

The second limitation lies in the lack of controls. In evaluating the effect of the bypass operation on the symptom of angina pectoris it is not practical to have a controlled study since this would require sternotomy and pericardiotomy in the randomized control patients too Control as regards the effect on long-term survival is however quite possible but it would require a large number of patients and a long follow-up and falls outside the scope of this study

ADDENDUM

Since the completion of this paper another 46 patients have been operated on with aortocoronary vein bypass for angina pectoris in this hospital. There have been no further deaths. This brings the total mortality of the operation down to less than 1 %

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CARDIAC ARRHYTHMIAS ELECTROLYTES AND DIGOXIN CONCENTRATION IN PLASMA AND URINE IN PATIENTS TREATED WITH DIGOXIN

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Abstract Cardiac arrhythmias, digoxin concentration in plasma and urine, digoxin and creatinine clearances, electrolytes in plasma and in erythrocytes, and subjective symptoms have been carefully studied for 5 consecutive days in 19 patients with definite or suspected digitalis intoxication. The digoxin treatment was discontinued during the observation period. Eleven controls without any signs of toxicity were similarly followed on unchanged maintenance dosage. All patients were independently classified as toxic or non-toxic from the follow-up of extended ECG recordings and subjective symptoms. In 9 definitely toxic patients plasma digoxin concentration of 3.1±0.7 ng/ml was found, as compared to 1.4±0.5 ng/ml for the 11 controls. In the suspect toxic group 1.5–3.9 ng/ml was found. The high digoxin level in the toxic group corresponds to a low digoxin clearance. In the toxic patients cardiac arrhythmias were related in most cases to a plasma digoxin level above 2.5 ng/ml and usually disappeared when the concentration had decreased below this. Suspect toxic patients, classified as probably non-toxic, and controls had with two exceptions plasma digoxin levels below 2 ng/ml. It is suggested that digitalis toxicity should be considered at plasma digoxin concentration above 2 ng/ml. It must be stressed that this limit is not absolute and is affected by among other things, disturbance of intra- and extracellular electrolytes.

Despite long experience with the digitalis glycosides, the frequency of patients with symptoms of toxicity seems to have increased during recent decades. In studies on hospitalized patients treated with digitalis the reported incidence of toxicity has ranged from 7 to 23% (?).

Several reasons have been suggested for the increased incidence of digitalis toxicity Major factors are the narrow therapeutic range of the glycosides and an increased use of diuretics which disturb the electrolyte balance the therapeutic dose at rapid digitalization, according to Lown and Levine (14) is about 63% of the toxic dose Similar values have been obtained for digoxin when given as maintenance treatment (15). Animal experiments have shown that approximately 50% of the lethal dose has been ingested when signs of digitalis toxicity appear

A promising approach to these problems appeared when clinically useful methods—a radioimmunoassay (17) and a modified rubidium (^{86}Rb) method (16)—for direct estimates of plasma digoxin concentration became available Several recent studies, using these techniques have shown significantly higher plasma digoxin levels in patients with signs of digitalis toxicity than in non-toxic patients treated with the glycoside. For a summary see Smith and Haber (18) In all investigations there is some overlap In only two studies (11 13) were the authors using a radioimmunoassay unable to differentiate between toxic and non-toxic patients

A main problem is the difficulty of defining the toxicity of digitalis ECG criteria often constitute the important basis for the diagnosis However a short routine ECG can give misleading results because rhythm disturbances may be of short duration, possibly intermittent, or unrelated to digitalis toxicity The purpose of the present investigation was to study arrhythmias in patients treated with digoxin and to correlate them in the first place to plasma digoxin concentrations Extended ECGs extracardiac symptoms and plasma digoxin concentration measured with an ^{86}Rb method have been carefully registered over a period of 5 con-

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Table I *Clinical data on the patients*

Pat no.	Age (y)	Sex	Diagnosis[a]	Extra-cardiac symptoms[b]	Diur	K subst.	Digoxin dosage (mg/d.)	Digoxin in plasma (ng/ml)	Digoxin clear (ml/min)	Creatin. clear (ml/min)
Toxic group										
1	79	♂	ASHD	A N V	+	+	0.25	3.2	77	24
2	79	♂	ASHD	–	–	–	0.375	2.6	45	NE
3	74	♀	ASHD+1+2	A N V	+	+	0.25	3 1	32	23
4	78	♂	HCVD	N V P	+	–	0.50	3.8	31	33
5	65	♀	RHD	A	–	–	0.25	3.2	20	51
6	77	♂	ASHD	–	+	+	0 125	3.8	10	8
7	89	♀	ASHD+3	A N V	+	+	0.25	3.9	39	30
8	71	♀	ASHD+4	A D	–	–	0.25	1 4	23	55
9	74	♀	HCVD+5	–	+	+	0 125	3 0	10	10
Suspect toxic group										
10	70	♂	ASHD+1+6	–	–	–	0.25	2.7	49	38
11	84	♂	ASDH	N	+	+	0.25	2.5	43	NE
12	61	♂	ASHD	N V D	+	+	0.375	1 7	66	NE
13	79	♂	ASHD+7	A N V	+	+	0.25	3 1	26	25
14	84	♀	ASHD	A D	+	+	0.25	[illegible]	29	37
15	62	♂	ASHD	–	+	–	0.375	2.2	42	60
16	76	♀	ASHD	A N V	+	+	0.375	3.9	42	42
17	84	♀	ASHD	–	+	+	0.25	2.6	44	42
18	51	♂	ASHD	P	+	+	0.375	1.5	89	86
19	67	♂	HCVD	A N V	+	+	0.375	[illegible]	14	9
Controls										
20	74	♂	ASHD+8		–	–	0.25	1.2	74	55
21	69	♂	ASHD+1+6		+	+	0.25	2.5	NE	40
22	69	♂	ASHD		–	–	0.25	0 7	108	69
23	62	♀	ASHD+1+3		+	–	0.25	1 2	60	57
24	85	♂	ASHD		+	+	0.25	2.1	32	45
25	77	♀	ASHD+3		+	–	0.31	1.0	60	65
26	63	♀	RHD		+	+	0.25	1 4	41	55
27	70	♂	ASHD		–	–	0.25	1 1	89	86
28	88	♀	ASHD+3		+	+	0.125	1.3	17	35
	71	♂	ASHD		+	+	0.25	1.6	25	35
	76	♂	ASHD		+	+	0.25	1.2	81	70

[a] 1=diabetes mellitus, 2=cancer mammae c met., 3=hyperthyroidism (treated), 4=hypertension 5=pyelonephritis chron., 6=phaeochromocytoma, 7=lymphosarcoma, 8=cancer hepatis.
[b] A=anorexia, N=nausea, V=vomiting, P=abdominal pain, D=diarrhoea.
Treatment with diuretics. +=treatment with furosemide in various dosage, except in case 18 in whom ethacrynic acid was used. In cases 21 and 23 complementary treatment with spironolactone was given.
Potassium substitution.
NE=not estimated

secutive days. Furthermore creatinine and digoxin clearances were followed as well as some extra- and intracellular electrolytes.

DESIGN OF THE EXPERIMENT

The present investigation involved patients with various signs indicating digitalis intoxication, and a control material. The patients classified as toxic were mostly admitted because of their toxic symptoms, but some were already in the hospital when signs of intoxication developed. Patients who had taken their last digoxin dose more than 24 hours before admission were excluded, as were those with acute myocardial infarction.

A preliminary classification of toxic or suspect toxic patients was performed by means of the case history and conventional preliminary 1-lead ECG registered shortly *after admission. In these cases digoxin therapy* was discontinued. The patients serving as matched controls were selected from the medical wards and were treated with their usual maintenance dose of digoxin throughout the investigation. They had no clinical or ECG signs of digitalis intoxication and went through the investigation as the other groups.

The case history was taken from a standardized printed questionnaire which enquired about symptoms that could be associated with digitalis toxicity, e.g. anorexia, nausea, vomiting, abdominal pain and diarrhoea. All patients were carefully followed in a standardized way for 5 days with

K/e (mEq/l)	K/i (mEq/l)	Na/i (mEq/l)	Electrolyte values diverging from normal range (mEq/l)
4.4	112	12.4	Cl/e 110
4.6	83	21.2	
3.6	93	11.1	Cl/e 88 Ca/e 5.5 Mg/e 1.4
3.7	117	23.6	
4.5	92	7.7	
4.5	101	14.0	
3.8	85	27.0	Na/ 128
4.8	79	9.8	
3.7	102	14.0	Na/e 137
4.5	84	26.4	Mg/e 1.5
5.0	89	12.4	Cl/e 97
4.4	105	19.4	
4.1	100	23.6	Cl/e 96 Ca/e 4.1
4.5	93	18.3	
4.5	106	10.4	
4.7	86	34.3	Cl/e 96
5.4	94	21.2	
4.8	91	17.3	Cl/e 97
5.6	89	21.2	
3.4	73	18.2	Cl/e 97 Na/e 136
4.6	102	9.3	Cl/e 97 Na/e 136
4.4	93	13.8	
4.7	90	13.2	Cl/e 111
4.7	84	29.4	Cl/e 111 Na/e 149
5.1	93	22.2	
4.3	101	15.6	
4.2	87	23.8	Mg/e 1.5
4.1	95	17.2	
4.2	88	16.8	
[illegible]	88	16.2	Mg/e 1.5

daily estimates of plasma digoxin, extra- and intracellular electrolytes, serum creatinine, and 24-hour creatinine and digoxin clearances. The first samples for estimating digoxin in plasma were drawn about 24 hours after ingestion of the last digoxin dose. In the control group the serial samples were always drawn 24 hours after the last dose given. Possible changes in the symptoms were specially asked for. A daily 12-lead ECG with a rhythm strip of 20 min duration was included.

When patient had gone through the whole program, one of us (P. O.), who did not know anything about the patient, made an independent evaluation of the 20-min ECG. For the final, retrospective classification, the occurrence of cardiac arrhythmias and their disappearance when discontinuing the digitalis treatment were considered together with the clinical picture.

Criteria for definite and suspected digitalis intoxication

1. *Definite digitalis toxicity.* (*A*) Marked extracardiac symptoms which could be related to digitalis toxicity. (*B*) Certain rhythm disturbances, paroxysmal atrial tachycardia with atrioventricular block, atrioventricular nodal escape rhythm, multifocal ventricular extrasystoles, ventricular bigeminy or trigeminy. 2nd degree AV block. For the diagnosis, *B* in most cases combined with *A*, should be present and should disappear when digitalis treatment is discontinued.

2. *Suspected digitalis toxicity.* (*A*) Extracardiac symptoms which could be related to digitalis toxicity. (*B*) Certain rhythm disturbances: sinus bradycardia, sinoatrial block. For the diagnosis, *A* or *B* should be present and should disappear when digitalis treatment is discontinued, or arrhythmia under 1*B* should be present but not disappear completely when digitalis treatment is discontinued. Also patients preliminarily classified as toxic or suspected toxic are included.

MATERIAL

Most of the patients with suspected or definite intoxication, according to the final classification, had been treated with an unchanged digoxin dose for more than one month, often for many years. One patient classified as suspected intoxication, had received the glycoside for only 8 days. The patients serving as controls had similarly been treated for a long time with an unchanged digoxin dose, except for two who had received the glycoside for only 8 and 9 days, respectively.

The digoxin dosage was 0.125–0.375 mg once a day except for one toxic patient, who received 0.5 mg daily. All patients were treated with the same brand of digoxin (Lanacrist® Draco, Lund, subsidiary to Astra, Sweden).

According to the final classification, 9 patients belonged to the toxic group, 10 to the suspect toxic group and 11 were accepted as controls. The first two groups comprised 8 females and 11 males; their ages ranged from 51 to 89 years (mean 74). The control group contained 4 females and 7 males, their ages varying from 62 to 88 years (mean 73).

The indication for digoxin treatment in most cases was congestive heart failure. Two of the controls were given digoxin for atrial fibrillation alone. In the group of definite and suspected intoxication, 15 patients had arteriosclerotic heart disease (ASHD), in one case combined with therapeutically corrected hyperthyroidism, 3 had hypertensive cardiovascular disease (HCVD), and one had rheumatic heart disease (RHD). In the control group 10 patients had ASHD, in three of them combined with treated hyperthyroidism without signs of activity, and one had RHD. Further details about the patients are given in Table I.

METHODS

Digoxin concentration in plasma

The method used is a modification of the [86]Rb method previously described in detail (4). For an analysis, 2 ml of plasma was extracted with 5 ml of newly distilled di-

chloromethane. After 10 min of centrifugation at about 1 900×g the plasma was socked off and 4 ml of the clear dichloromethane phase was transferred to new tubes. These were put into a water bath and placed in a fume cupboard After about 30 min at 50°C, the temperature was increased to 70°C to ensure a complete evaporation of the extracts. The residue was then dissolved in 1 ml of NaCl glucose solution, and 0.5 ml of washed packed red cells was added to each sample. This was followed by a preincubation in a water bath at 37°C for 2 hours. After cooling the samples in ice water for a few minutes, about 6 µCi of ^{86}Rb was added and the incubation at 37°C continued for another hour Thereafter the samples were centrifuged The supernatant was sucked off and the cells were washed twice with about 10 ml of saline solution.

Finally the γ-activity of the washed and packed erythrocytes was registered in a well crystal. I each analysis, standards containing known amounts of digoxin and samples without digoxin were run parallel with the patient samples, performed in triplicate.

The S D of the method is 0 1 ng digoxin/ml plasma within the triplicate determinations as well as between these triplicates performed on different days.

Digoxin concentration in urine

The procedure for urine assay was mainly as described for plasma samples. In the standard series including zero samples, however normal urine, containing no digoxin or other heart glycosides, was used instead of glycoside-free plasma. As the digoxin content in patient urine was mostly rather high, a screening assay was always made in order to choose convenient volume for each sample not containing more than 6 ng digoxin. This was done because the standard reference curve could be regarded as straight only up to a concentration of 3 ng/ml In most cases 50 µl of urine was used for the assay This volume was diluted up to 2 ml with normal urine thereafter the assay was run identically as for plasma

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al amount of digoxin excreted daily in urine during days was estimated I toxic and suspect toxic patients, the mean plasma digoxin concentration of the separate days was known and the 24-hour digoxin clearance was calculated. In the control patients, the daily fluctuation in plasma levels after dose administration was taken into consideration. Planimetric calculations showed that a value 0.3 ng/ml higher than the mean of the plasma concentrations enclosing the 24-hour period should be used.

Creatinine was estimated by the method described by Jaffé and modified by Henry (1.), eliminating non-creatinine chromogens V lues given in Table I for both digoxin and creatinine clearances are the mean of repeated 24-hour clearances.

Electrolytes in red blood cells and in plasma

A direct method, described by Bengtsson et al. (3), was used for the determination of electrolytes in erythrocytes. Blood samples of 5-8 ml were drawn from the antecubital fossa, in most cases without stasis. In a few patients a slight stasis was applied during the venous puncture thereafter blood was drawn without stasis. Coagulation was inhibited by sodium heparin. The blood was immediately centrifuged at 2000×g for 3 min plasma was then removed. The erythrocytes were washed twice in an equal volume of choline Ringer solution (choline chloride 146 $MgCl_2$ 1 $CaCl_2$ 1 NaCl 5 ortophosphoric acid 2.5 mM tris buffer to pH 7 4). The erythrocyte suspension was centrifuged at 2 000×g for 3 min. After each washing the choline Ringer solution was removed The erythrocytes were haemolyzed and diluted with distilled water For sodium determination 5 ml distilled water was added to 100 µl erythrocytes. For potassium, the samples were further diluted 1 11 Each sample was analysed four times in a Unicam SP 90 flame photometer All figures were corrected for trapped extracellular fluid and for water lost during washing and centrifugation according to Bengtsson et al (3).

The S.D of the method, within four determinations on the same material, was 0.3 mEq sodium and 2.1 mEq potassium per l RBC. The S.D of determinations made on different days gave similar results, 0.3 mEq sodium and 2.4 mEq potassium respectively

The range of *intracellular sodium* concentration (Na/l) in 30 normal subjects (15 males and 15 females) has been found to be 5 7 10.5 mEq/l of RBC (mean 8.0). The range of *intracellular potassium* concentration (K/l) was 84–112 mEq/l of RBC (mean 98).

Sodium, potassium, and calcium in serum were determined with flame photometry For estimation of magnesium in serum, a spectrophosofluorometric method was used

Electrocardiography

A preliminary conventional 12-lead ECG was registered at the beginning of the investigation. As a routine this ECG was registered for 10 sec in patients with regular rhythm and for 40 sec in patients with arrhythmias. The information from the ECG was considered in the preliminary classification of the patients.

The patients were followed up for 5 days with a daily 20-min ECG, registered continuously with the patients in complete bed rest. The ventricular rate was estimated by counting all the QRS complexes during the last minute of registration.

RESULTS

Plasma digoxin concentration and urinary excretion of digoxin

No correlation was found between the dose administered and the plasma digoxin levels registered Most of the patients 19 of 30 had been treated with 0.25 mg once daily They showed plasma digoxin levels ranging from 0 7 to 3.9 ng/ml that correlated significantly ($p<0\ 01$) with the digoxin clearance. In the group of definite intoxications 2 of 9 patients had only received 0.1.5 mg digoxin daily Despite the low dose their plasma levels were 3.8 and 3 0 ng/ml respectively

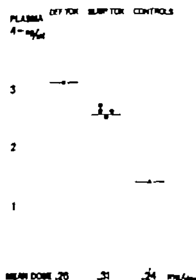


Fig. 1. Plasma digoxin levels and the mean digoxin dosage in the definitely toxic, suspect toxic, and control patients.

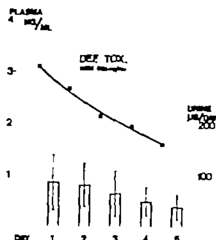


Fig. 3. Mean plasma digoxin levels (■—■) and urinary excretion of digoxin for the 5 days of observation in the patients with definite digitalis toxicity (mean ±S.D.).

Patients with definite digitalis intoxication had with one exception, high plasma digoxin levels 2.6–3.9 ng/ml 24 hours after the last dose (Table I Fig. 1). Most controls had plasma values well below 2 ng/ml (mean 1.4). Only two of them showed values above 2 ng/ml. The patients with suspected digitalis intoxication had plasma digoxin levels ranging from 1.5 to 3.9 ng/ml.

The control patients receiving an unchanged digoxin maintenance dose during the 5 days showed no significant change in mean plasma digoxin levels during the study (Fig. 2). The weighed S.D. between examinations was 0.2 ng/ml. In the other patient groups plasma digoxin concentration declined about linearly when the digoxin therapy was discontinued (Figs. 3 and 4).

In the control patients a mean of 129 µg digoxin was found daily in the urine (Fig. 2) which is 54% of the average maintenance digoxin dose. Great variations were seen between the patients, ranging from 28 to 70% of the daily dose. No significant difference was found in urinary digoxin excretion

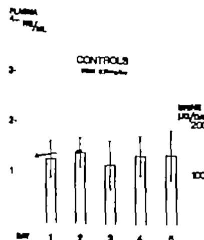


Fig. 2. Mean plasma digoxin levels (▲—▲) and urinary excretion of digoxin for the 5 days of observation in the control patients (mean ±S.D.).

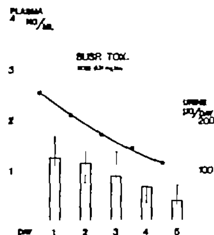


Fig. 4. Mean plasma digoxin levels (●—●) and urinary excretion of digoxin for the 5 days of observation in the patients with suspected digitalis toxicity (mean ±S.D.).

Table II. *Cardiac arrhythmias and their correlation to plasma digoxin levels*

Digoxin/Pl. at *App* =plasma digoxin level when an arrhythmia was observed initially When two values are given, the arrhythmia did not disappear In these cases, the highest and lowest digoxi values registered are given. *Disapp* =plasma digoxin level when an arrhythmia disappeared

Type of arrhythmia	Definite toxic: Case no.	Disappeared in case no	Digoxin/Pl. at App.	Digoxin/Pl. at Disapp	Suspect toxic: Case no	Disappeared in case no	Digoxin/Pl at App	Digoxin/Pl at Disapp.
PAT with block	6	6	3.8	4 0[a]				
AV nodal escape rhythm	2		2.6	1.5				
	4	4	3.8	3 6[b]				
	1	1	3 9	.3				
Ventricular bigeminy or trigeminy	3	3	3.2	2.1				
	5	5	3 1	1 6				
	7	7	3.2	1.8				
Multifocal ventr extra-					12	–	1 7-0.7	
systoles	9	9	3.0	2.5	18		1.5-0 7	
2nd degree AV block	8	8	1 4	0.9				
Sinus bradycardia					19	19	2.7	1.8
SA block					11	11	2.5	1 4
Sinus arrhythmia	1	–	3.–1.5		10	–	2.7 1.2	
Atrial extrasystoles	1	–	3.2 1.5		10	–	2.7 1.2	
	3		3 1-1.6		13		3 1–.2	
	6		4 0-2.0		17		2.6-1 4	
	8		1 4-0 7					
Unifocal ventr extra-					10		2.7-1.2	
systoles (<5/min)	4	–	3.8–.3		11	–	2.5-1.2	
					14		2.6-1 1	
					16		3 9-2.5	
					17		2.6-1 1	

A 1st degree AV block persisted.
A atrial fibrillation with a ventricular rate below 50/mi persisted.

from one day to another In the toxic and suspect ...ic patient group the total daily amount of ... found in the urine fell continuously ... the observation period (Figs. 3 and 4). ... data for these groups are not comparable with those for the control patients who were still on digitalis treatment

Renal function was impaired in many of these old patients especially in the toxic group (Table I) There was a good correlation between creatinine and digoxin clearances =0.83 $p<0.001$ (Fig. 5) However discrepancies were noted in some patients. The digoxin clearance for the toxic group was 26±12 ml/min and for the controls 59±30 ml/min, the suspect toxic group was intermediate 44±21 ml/min The difference between the toxic and control patients is significant ($p<0.01$).

Electrocardiographic findings

The basic rhythm was found to be sinus rhythm in 6 of 11 patients in the control group the remaining controls had atrial fibrillation. In the toxic and suspect toxic group sinus rhythm was observed in 9 patients nodal rhythm in one and atrial fibrillation in 9

In the toxic group the following rhythm disturbances were seen (Table II) paroxysmal atrial tachycardia with AV block—PAT with block (1 patient) AV nodal escape rhythm (2 patients) second degree AV block (1 patient) ventricular bigeminy or trigeminy (4 patients) and multifocal ventricular extrasystoles (1 patient) In the cases of bigeminy or trigeminy the ventricular extrasystoles were unifocal in 3 patients and multifocal in one. In two patients (cases 4 and 6) the arrhythmias (AV nodal escape rhythm and PAT with block) disappeared as early as on the second day of observation despite persistently high plasma digoxin levels (3 6 and 4 ng/ml respectively) but these arrhythmias were followed by an atrial fibrillation with a ventricular rate below 50/min (case 4) and a 1st degree AV block (case 6) These two changes were both considered

Controls			
Case no.	Disappeared in case no	Digoxin/Pl. at	
		App.	Disapp.
[illegible]	30	2.3–1.8	
[illegible]		1.6–1.3	
[illegible]		0.8	1.3
[illegible]		2.7–2.2	
[illegible]	–	1.1–0.9	
[illegible]		1.6–1.3	
[illegible]		1.3	
[illegible]		1.6	
–			

to be digitalis-induced as the bradycardia disappeared and the PR intervals gradually shortened during the following days with decreasing plasma digoxin levels.

All the three patient groups displayed sinus arrhythmia, atrial extrasystoles and unifocal ventricular extrasystoles (less than 5/min). These rhythm disturbances were seen within a wide range of plasma digoxin levels and they did not change significantly in the toxic and suspect groups when the digitalis therapy was discontinued. Therefore they were considered to be unrelated to digitalis toxicity.

Ten patients belonged to the group of suspected digitalis intoxication. One of these had sinoatrial block (SA block) and one had sinus bradycardia (heart rate below 50/min). These two rhythm disturbances disappeared when digitalis was discontinued. Six patients belonging to the group showed no arrhythmia which could be attributed to digitalis toxicity. In two other patients, multifocal ventricular extrasystoles were observed. However, they did not disappear or change significantly in frequency when digitalis was discontinued, even though the plasma digoxin fell to low values (0.7 ng/ml). Therefore it was concluded that the multifocal ventricular extrasystoles in these two cases were not related to digitalis intoxication. In the 11 controls no arrhythmia attributable to digitalis toxicity was observed (Table II).

The details regarding the relation between cardiac arrhythmias and plasma digoxin levels are given in Table II.

Extra- and intracellular electrolyte concentrations

In the control patients, the mean concentration of Na/l for 5 days was found to range from 9.3 to 29.4 mEq/l with a mean of 17.8 mEq/l. For the suspect toxic and toxic groups the initial means were 20.4 (range 10.4–34.3) and 15.6 (range 7.7–27.0) mEq/l.

The range of K/l in the control group was 75–102 mEq/l (mean 90.5). For the suspect toxic group the initial mean was 93.3 (range 84–106) mEq/l. For the toxic group it was 95.8 (range 79–117) mEq/l.

The *extracellular sodium* concentration (Na/e) ranged from 128 to 149 mEq/l (mean 140.4). There was practically no difference between the means for the three groups. In the control group 2 patients had a slight hyponatraemia (136 mEq/l) and one a slight hypernatraemia (149 mEq/l). In the group of

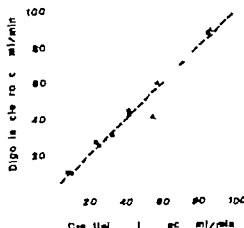


Fig. 5. The correlation between digoxin and creatinine clearances in definitely toxic (■), suspect toxic ( ), and control (▲) patients (r=0.83).

digitalis intoxications, 137 mEq/l was registered in one patient and 128 mEq/l in another Hypopotassaemia (3.2 mEq/l) was found in one patient in the control group Increased extracellular *serum potassium* (K/e) 5 1 and 5 4 mEq/l was found in 2 patients in the control group and in 3 in the suspect toxic group (5 6 5 4 and 5 0 mEq/l) Mean K/e was somewhat lower in the toxic group than in the others ($p<0.05$) but still well within normal limits. Extracellular *serum calcium* (Ca/e) was slightly increased (5.5 mEq/l) in one patient in the toxic group and slightly decreased (4 1 mEq/l) in one subject in the suspect toxic group Extracellular *serum chloride* (Cl/e) was slightly elevated (111 mEq/l) in one control patient and slightly decreased in 7 patients Extracellular *magnesium* (Mg/e) was slightly decreased in 4 patients (1 4–1.5 mEq/l), otherwise normal.

The intra- and extracellular electrolyte concentrations showed no systematic changes during 5 days of observation.

DISCUSSION

In the evaluation of plasma digoxin as a tool in the diagnosis of digitalis intoxication the criteria for toxicity are evidently of decisive importance In previous investigations, the classification of digitalis toxicity has been based largely on ECG findings. Unfortunately there is no cardiac arrhythmia specific for digitalis toxicity Furthermore a transitory arrhythmia, possibly related to digitalis toxicity may be missed in the registration only short conventional routine ECGs or rhythm stips. At the same time such an arrhythmia can be judged erroneously as "disappearing" if it is missing in a subsequent short serial recording Therefore in the present investigation, the ECGs were registered 20 min daily for 5 days

Even if carefully studied cardiac arrhythmias might not be sufficient to determine the diagnosis of digitalis toxicity Pronounced subjective symptoms indicating toxicity were therefore taken into account as well These symptoms must be appraised in relation to the whole clinical picture

Because ECG findings play such an important role for the classification of digitalis toxicity it is of particular interest to correlate rhythm disturbances and their possible disappearance to the plasma digoxin levels (Table II) PAT with block AV nodal escape rhythm ventricular bigeminy or trigeminy and multifocal ventricular extrasystoles were observed in 8 patients and were considered to be related to digitalis toxicity When these arrhythmias were observed initially the plasma digoxin level was more than 2.5 ng/ml in all 8 patients When they disappeared the plasma digoxin concentrations were 2.5 ng/ml or less in 6 of the 8 patients In the remaining two patients other manifestations of digitalis toxicity persisted until the digoxin level had decreased (Table II).

The eight toxic patients discussed so far had plasma digoxin levels well above 2.0 ng/ml Case 8 however classified as toxic because of a 2nd degree AV block and extracardiac symptoms which disappeared when digitalis therapy was discontinued had a plasma digoxin concentration usually regarded as non-toxic (1 4 ng/ml). It is interesting to note that, despite a normal K/e value she had low intracellular concentrations of this ion (Table I). Among the extracardiac symptoms, this patient suffered from a marked diarrhoea that had started about one week before admission to hospital It seems probable that this was an important reason for her electrolyte disturbance triggering toxic cardiac symptoms at a non-toxic plasma digoxin level

Ten patients had rhythm disturbances and extracardiac symptoms which placed them in the group of suspected digitalis toxicity Two of these patients might be regarded as toxic since their rhythm disturbances—AV block (case 11) and sinus bradycardia with a ventricular rate below 50/min (case 19)—disappeared when digitalis therapy was discontinued (Table II) Another two of these patients (cases 12 and 18) were probably non-toxic because their arrhythmia (multifocal ventricular extrasystoles) persisted although they received no digitalis It is of particular interest that the plasma digoxin level was above 2 ng/ml in the two patients considered to be toxic and well below this level in those considered non-toxic. Thus the digitalis estimate agrees with and strengthens the clinical evaluation. Six of 10 patients in the suspect toxic group cannot clinically be further divided into toxic or non-toxic since their extracardiac symptoms and rhythm disturbances did not change significantly although the digitalis therapy was discontinued In two of them (cases 13 and 16) this might be because that even 5 days was too short an observation period Their plasma levels on admission were

3 and 3.9 ng/ml respectively and had decreased to 2.2 and 2.5 ng/ml respectively by the last day They might thus have been toxic despite persistent symptoms and arrhythmias In the remaining 4 patients the plasma levels were on the whole intermediate between those registered in the toxic group and in the controls (2.1–2 7 ng/ml).

Multifocal ventricular extrasystoles are of particular interest since they were observed in 3 patients at a plasma digoxin level of 3 0 1 7 and 1.5 ng/ml respectively (see above). The multifocal ventricular extrasystoles disappeared in only the first of these three patients In the other two the *ectopic beats remained for the 5 days although the* plasma digoxin level had decreased to 0 7 ng/ml It seems reasonable to conclude that multifocal ventricular extrasystoles are an uncertain indicator of digitalis toxicity Fogelman et al. (11) who found no correlation between plasma digoxin level and digitalis toxicity seem to have based the diagnosis of digitalis toxicity on multifocal ventricular extrasystoles in 5 of 18 cases, which seems to be dangerous.

The plasma digoxin estimate seems to be of value in the diagnosis of digitalis intoxication The present data indicate that digitalis intoxication is highly probable *when the plasma digoxin level is above 3* ng/ml Furthermore a plasma digoxin level below 2 ng/ml can be considered as non-toxic in patients with normal electrolyte values. According to our experience plasma digoxin levels above 2 ng/ml should arouse suspicion of digitalis intoxication even in the absence of clinical signs of toxicity These patients should be carefully controlled and a reduction of their digoxin dosage should be considered as exemplified by one control patient (case 21). During the 5 days he had a mean plasma digoxin level of 2.5 ng/ml. On unchanged digoxin dosage he developed a definite digitalis intoxication a few weeks later

The electrolyte balance is one of the factors influencing a patient s sensitivity to digitalis glycosides. It is well known that digitalis preparations have an inhibitory effect on the active cation transport through e.g. the red cell membrane. In the present material 26 patients (87%) were found to have abnormally high concentrations of intracellular sodium (Table I) Our results agree with those of Tosa (19) who found that 88% of a group of rapidly digitalized patients had increased Na/I concentrations Hypertension may have contributed to the intracellular hypernatraemia in some of the patients this condition being capable of inducing a high sodium concentration in red blood cells No significant correlation was found between digoxin concentration in plasma and sodium concentration in RBC

Tosa (19) found decreased K/I concentration in 78% of digitalized patients. In our material only 6 patients of 30 (20%) were found to have decreased potassium concentrations in RBC The lower incidence of hypopotassaemia as well as the lack of correlation between the plasma digoxin concentration and K/I in our material might be due to a *generous potassium substitution No significant* correlation was found between sodium and potassium concentrations in the RBC

Digoxin is primarily excreted unchanged in the urine Bloom and Nelp (8) and later Doherty and Flanigan (10) using tritiated glycoside, showed that digoxin clearance corresponded well to the creatinine clearance This was confirmed in the present study (Fig 5). Contrary to our findings a poor correlation between the two clearances has been reported recently with digoxin clearances consistently lower than those of creatinine The discrepancy between our results and those of Baylis et al (1) probably has methodological reasons The radioimmunoassay used by these authors was shown recently to be influenced by substances other than digoxin (7). In a study on the reliability of radioimmunoassay for direct estimates of urinary digoxin concentration, we found that urine from subjects not treated with digoxin or other heart glycosides might contain more than 6 ng "digoxin units"/ml Hormone metabolites with a steroid nucleus are probably the reason for these erroneously elevated values The ⁸⁶Rb method yields reliable urine glycoside values

The daily excretion of digoxin fell after digoxin treatment had been discontinued in the patients with definite or suspected digitalis toxicity In the definite toxic group the mean urinary excretion was somewhat less than in the group of suspected toxicity despite higher plasma digoxin values probably due to a more impaired renal function (Table I)

The control patients e creted I the urine on an average 54% of the daily maintenance digoxin dose There were large interindividual differences—28–70% of the digoxin dose ingested—I most cases correlated to the renal function It

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REGISTRATION OF SINUS NODE RECOVERY TIME IN PATIENTS WITH SINUS RHYTHM AND IN PATIENTS WITH DYSRHYTHMIAS

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Abstract. Sinus node recovery time (SRT) after rapid atrial pacing has been recorded in 66 patients 28 with coronary heart disease 11 with advanced AV block, 10 with sick sinus syndrome and 17 with paroxysmal tachyarrhythmias. In patients with normal functioning sinus node SRT was related to the basal heart rate. On an average SRT was 130 % of the basal P-P interval with an upper limit of 160 % In patients with presumed normal atrial function the mean SRT was found to be 1080 msec, with an upper limit of 1500 msec. This corresponds with previously published observations. In all 5 patients examined, β-receptor blockade (propranolol 5 mg i) prolonged SRT The prolonged SRT was related to sinus bradycardia. Verapamil (Isoptin® 5 mg i.) had no effect of SRT in the 7 patients examined The observation of an SRT of more than 1500 msec indicates a poor sinus node function. Recording of a normal SRT however cannot exclude a sinus node dysfunction, as normal SRT is occasionally found even in patients with a clinically proved dysfunction.

The clinical manifestations of sinus node dysfunction include sinoatrial block, sinus arrest sinus bradycardia and tachybradycardia syndrome. In patients in whom these disturbances are recorded or suspected a complete evaluation of the sinus node function is warranted Methods for examination of the sinus node function are at present insufficient It has not been possible to record either ECGs from the sinus node in patients or the sinoatrial transmission time. The sinus node automaticity however can be tested by recording the postpacing suppression. This interval is called the sinus node recovery time (SRT) SRT can be recorded after induction of a supraventricular extrasystole by atrial pacing (4) or more conveniently after abrupt cessation of atrial overdrive pacing (3 4 6)

The purpose of the present study has been to record SRT in patients with a presumably normal sinus node function, and in patients with clinical signs of sinus node dysfunction

METHODS

Atrial pacing was performed with bipolar pacing electrodes introduced pervenously from the right cubital or the femoral vein. The pacing electrodes were placed adjacent to the sinus node in the upper posterolateral part of the right atrium. Usually two pacing catheters were introduced, and intraatrial P waves were recorded from one electrode, while the other was used for pacing. I the first 10 patients examined, however only one electrode was used, and the P waves had to be detected from the extremity leads I II III and the precordial lead V

Medtronic external pacemaker type 5837 was used. The electrode position was accepted when pacing threshold was 3 mA or less. The atria were paced with an impulse twice threshold value at rates of 100 120, and 140/min At each rate pacing was discontinued after 60 sec spontaneous rhythm recorded for 20 sec and pacing resumed for another 60 sec. Thus 2 observations were made for each pacing rate and the longest SRT observed after each pacing rate was used for further analyses The interval between the fifth and sixth normal P wave after trial pacing was found to be representative for the basic atrial rhythm in most of the patients and t was sed in calculations as the patients basal P P interval When atrial rhythm was irregular the verage of the fiv following P-P interval was used. P-P intervals of 5000 msec or more were defined as sinus arrest ECG was recorded on Mingograph, paper speed 50 mm/sec, and the intervals were measured on the ECG paper For the statistical analysis the paired *t*-test was used

MATERIAL

SRT was recorded in 66 patients, 28 of whom had routine right heart catheterization in the preoperative investigation for coronary heart disease (CHD) Eleven patients with advanced AV block were xamined in connection with registration of His bundle potentials or introduction of temporary pacemaker electrode. Seventeen patients

Table I *Sinus node recovery time in patients with coronary heart disease*

	SRT (msec)			SRT (% of basal P P interval)		
	Atrial pacing rate			Atrial pacing rate		
Pat. no	100	170	140	100	120	140
1	1 130	1 180	1 150	138	128	121
	1 140	1 190	1 130	119	118	117
3	1 020	1 220	1 150	136	153	129
4	1 000	1 000	980	135	137	120
5	1 160	1 160	1 190	134	129	~
6	920	950	900	11	170	112
7	1 170	1 030	1 010	124	119	119
8	1 000	950	950	133	177	179
9	1 150	1 040		128	135	~
10	1 260	1 230	1 120	148	149	127
11	1 100	1 070	980	130	124	114
1	1 120	1 160	1 200	1_5	126	130
13	980	920	1 040	177	125	137
14	1 030	1 080		143	137	~
15	1 280	1 230	1 360	145	158	134
16	960	930	950	137	137	138
17	860	890	1 030	125	127	147
18	800	800	670	116	133	114
19	690	780		111	131	
20	~	830	820		143	138
1	770	980	830	114	126	134
22	1 390	1 380	1 280	136	134	170
_3	1 340	1 200	1 220	137	176	1_4
4	1 470	1 440		128	130	
_5	1 330	1 090	1 330	123	91	139
26	1 160	1 150	1 700	145	137	140
27	1 190	1 120	1 020	147	143	142
28	1 080	1 170	1 150	159	142	128
Mean	1 090±36	1 070±30	1 070±34	131±2	131±_	128±2

were examined due to episodes of paroxysmal tachyarrhythmia and 10 for suspected sinus node dysfunction.

The effect of β-receptor blockade and of erapamil on ST a xamined in 5 and 7 patients respecti ely with *ID and presumabl) normal sinus node function*

RESULTS

Pat ent ithout ymptoms of sinu node d function

In the 28 patient with CHD and presumably normal sinus node function average SRT was 1 060 msec After an atrial pacing rate of 100/min mean SRT was 1 090 msec after a pacing rate of 140/min mean SRT was 1 070 msec These differences were however not significant (Table I) Two patients (nos 10 and 18) who developed anginal pain during atrial pacing at a rate of 140/min had shorter SRT at that rate. *A positive correlation was found between* SRT and the basal endogenou P P interval (after a pacing rate of 100/min $R = 0.88$ after a pacing rate of 170/min, $R = 0.79$ after a pacing rate of 140/min, $R = 0.87$) The mean SRT was about 130% of the basal P P interval at all pacing rates

Patients with AV block

Eleven patients with advanced AV block were examined. One of them had a congenital and 10 an acquired AV block (Table II) The mean SRT was 1 070 msec after a pacing rate of 100/min and 1 190 msec after a pacing rate of 140/min These differences were not statistically significant Mean SRT *for all observations was 1 080 msec In these patients* the basal P P intervals were often difficult to assess due to irregular atrial rhythm In 4 patients P P intervals were shorter if a QRS complex appeared inbetween the two P waves In one case P waves tended to follow immediately after the start of QRS complexes probably due to *supraventricular extrasystoles and not to retrograde AV conduction* In 6 patients the longest P P interval did not follow immediately after termination of the atrial

Table II. *Sinus node recovery time in patients with advanced AV block*

	SRT (msec)			SRT (% of basal P-P interval)		
	Atrial pacing rate			Atrial pacing rate		
Pat. no.	100	120	140	100	120	140
1	1 140	1 170	1 240	146	131	141
2[a]	990	1 050	1 100	139	146	153
3	1 340	1 340	980	146	111	97
4[a]	1 40	1 200	1 320	1 4	108	123
5	1 300	1 200	1 140	129	124	114
6[d]	1 060	1 040	1 140	117	118	127
7	1 180	1 140	1 380	120	114	138
8	1 200	1 030	1 180	126	118	113
9	1 200	930	840	160	122	100
10[a]	1 070	1 410	1 460	135	178	185
11	1 160	1 320	1 360	129	145	166
Mean	1 170±30	1 170±50	1 190±60	134±4	129±6	132±8

Shorter P-P intervals with time relation to ventricular systole. Unpredictable variations in P-P interval longest spontaneous P-P interval 1 700 msec. Congenital AV block. Supraventricular extrasystoles.

pacing in one or more registrations. One of these patients (no 3) had on one occasion a spontaneous P-P interval of 1 700 msec. In the other patients all spontaneous P P intervals were less than 1 500 msec. In the patients with AV block SRT was on an average 131% of the basal P-P interval.

Patients with sick sinus syndrome

Ten patients with a clinical diagnosis of sick sinus syndrome were examined. The diagnosis was made before the SRT examination and was based on history and ECG recordings. Duration of symptoms and relevant clinical data are listed in Table III. The group included patients with marked tendency to sinus arrest as well as patients with moderate symptoms due to intermittent sinoatrial block. Eight of the patients had symptoms which warranted pacemaker implantation

In this group SRT varied greatly between patients and in many of them between registrations (Table IV). In of the 10 patients SRT was shorter than 1 500 msec in all registrations. In one of them however P P interval of more than 1 900 msec duration were observed during the next 8 sec after discontinuation of atrial pacing. In 6 cases long P-P intervals, even sinus arrest were observed a few seconds after atrial pacing as shown in Fig. 1. When episodes of sinus arrest (SRT>5 sec) were excluded a mean SRT of 1 780 msec was observed. Sinus arrest was seen in patients after pacing at a

Table III. *Clinical data on patients with sick sinus syndrome*

Pat. no.	Age (y)	Symptoms	Duration (y)	Tachyarrhythmias	AV block	Permanent pacemaker
1	70	Congestive heart failure	1	Parox. flutter	2:1	Yes
2	64	Dizziness	3			Yes
3	53	Dizziness	3	Parox. flutter		Yes
4	68	Dizziness	20	Parox. nodal tach		N
5	76	CHD	1	Parox. atrial tach.		Yes
6	80	Dizziness	9			Yes
7	77	Dizziness	1		Atrial pacing 120 Wenckebach	No
8	64	Dizziness, syncope	14	Parox. flutter	Atrial pacing 100-Wenckebach	Yes
9	53	Syncopes	6	Parox. flutter		Yes
10	64	Dizziness	6			Yes

Table IV *Sinus node recovery time (msec) in 10 patients with sick sinus node*

Pat. no.	Atrial pacing rate 100	120	140
1	1 420	–	Sinus arrest
	4 600		1 350
2	Sinus arrest	–	Sinus arrest
			Sinus arrest
3	–	Sinus arrest	1 650
		Sinus arrest	–
4	1 550	1 320	3 800
	1 700	1 430	4 400
5	800	4 500	940
	2 150	1 000	1 500
6	1 360	1 320	1 360
	1 360	1 200	1 200
7	1 220	1 050	1 100
	1 600	1 580	1 500
8	3 260	Sinus arrest	Sinus arrest
	2 730	Sinus arrest	Sinus arrest
9	1 170	1 015	1 150
	1 370	1 120	1 070
10	Sinus arrest	Sinus arrest	Sinus arrest
	Sinus arrest	Sinus arrest	Sinus arrest
Mean	1 870+/−270	1 550+/−330	1 920+/−30

In one or more of the registrations one of the following P P intervals was longer than the SRT
Episodes of sinus arrest (SRT>5000 msec) have been excluded.

rate of 100/min and in 4 patients after pacing at a rate of 140/min

Patients with paroxysmal tachyarrhythmias
Seventeen patients with paroxysmal tachyarrhythmias were examined (Table V) Fifteen had SRT shorter than 1 500 msec at all pacing rates. Two of the patients (nos 4 and 8) both with CHD had SRT of 1 500 msec or more Patient 8 had left ventricular aneurysm and paroxysmal ventricular tachycardia. During 2 weeks observation in a CCU sinus arrest, SA block or extreme bradycardia had never been observed In patient 4 who had had paroxysmal supraventricular tachycardia for many years one possible episode of sinus arrest had been seen on the oscilloscope screen during observation in a CCU

Irregular atrial rhythm after termination of atrial pacing was very common in these patients usually due to supraventricular extrasystoles in some cases to ventricular extrasystoles and retrograde P waves In one patient atrial pacing was followed by a supraventricular tachycardia for a few seconds. In 6 patients the longest P P interval did not follow immediately after termination of the atrial pacing in one or more of the registrations.

Effect of drugs on SRT
In 5 CHD patients SRT was recorded 10 min after β-receptor blockade (propranolol 5 mg i.v.) SRT increased from 1080+/−40 to 1300+/−50 msec and this increase was statistically significant (p=0 001) This prolongation of SRT corresponded to the bradycardia induced by the β-receptor blockade The basal P P intervals increased from 830 to 950 msec The SRT as a percentage of the basal P P intervals was unchanged (131% before, 133% after propranolol).

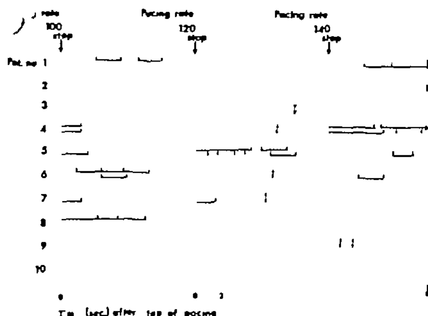


Fig 1 Rhythm diagram in patients with sick sinus node. Atrial rhythm during the first 8 sec after atrial pacing (pacing rates 100 120, 140/min) |=P waves, —=P-P intervals longer than 1 900 msec.

Table V *Sinus node recovery time in patients with tachyarrhythmias*

PAT = paroxysmal tachyarrhythmia, PAF = paroxysmal atrial fibrillation, VES = ventricular extrasystole, PVT = paroxysmal ventricular tachycardia, AES = atrial extrasystole

Pat. no.	Age (y.)	Type of tachyarrhythmia	SRT (msec) Atrial pacing rate 100	120	140	SRT (% of basal P-P interval) Atrial pacing rate 100	120	140
1	50	PAT	1 050	980	970	128	120	121
2	82	PAT	1 040	1 300	1 340	102	146	146
3	45	PAF	1 220	1 040	–	160	135	
4	73	PAT	1 500	1 500	1 300	142	130	140
5	59	PAT	1 000	940	1 070	119	122	179
6	55	PAT	1 000	950	880	113	102	105
7	31	VES	1 160	1 190	1 160	135	138	132
8	62	PVT	1 550	1 820	–	115	127	–
9	64	VES	1 050	1 060	980	135	137	126
10	62		1 260	1 350	1 150	136	132	125
11	54	AES Prox. AV block I	1 020	1 060	1 000	120	127	111
12	67	PVT	1 300	1 250	1 130	117	120	140
13	66	PAT	1 460	1 200	1 120	130	124	108
14	55	PAF	1 300	1 320	1 380	137	143	141
15	67	PAT	1 460	1 260	1 250	127	107	119
16	69	PAT	970	1 080	940	137	154	138
17	20	PAT	800	900	900	129	120	155
Mean			1 190+/−50	1 190+/−60	1 110+/−40	129+/−3.0	128+/−3.0	129+/−4.0

In 7 CHD patients SRT was recorded 10 min after verapamil (Isoptin® 5 mg i v) No significant effect was observed on the SRT The basal heart rate was unchanged SRT was 1 150 msec before and 1 180 msec after verapamil The difference was not statistically significant. SRT as a percentage of the basal P P interval was 134 before as well as after verapamil.

DISCUSSION

Recordings of SRT are easy to perform and can be done in connection with other invasive diagnostic procedures. It is advantageous to ha e an intraatrial ECG for recording of P waves because P waves in standard ECG leads sometimes are obscured by QRS or T waves Atrial extrasystoles are also usually easier to detect from an intraatrial recording In many patients a different duration of SRT can be observed from one registration to another and therefore more than one recording should be taken in each case.

In patients with no clinical dysfunction of the sinus node w found an average SRT of 1 080 msec and in no case an SRT longer than 1 500 msec These figures may be taken as normal values for SRT On an average SRT was 130% of the basal P-P interval and in no case more than 160% Our observations correspond fairly well with previously published results (1 3 5 6). In some of the patients with clinically proved poor sinus node function we have recorded a normal SRT on one or more occasions The recording of a normal SRT therefore does not exclude the possibility of a poorly functioning sinus node In some instances the following spontaneous P P intervals are longer than the SRT and may exceed 1 500 msec We have observed that even sinus arrest may occur a few seconds after atrial pacing.

It was previously found by others (3 4) that the duration of SRT normally is not related to the atrial pacing rate. Mandel et al. (3) however observed a reduction in SRT after atrial pacing rates of more than 150 W made th same observation of shortened SRT in patients, who developed angina pectoris at atrial pacing rates of 140/min Narula et al (4) observed that SRT lengthened as the pacing rate increased in patients with sinus bradycardia. In the present study sinu arrest occurred more frequently after rapid atrial pacing than after atrial pacing at a moderate rate in patient with sick sinus syndrome

To our knowledge the effect of β-receptor blockade on SRT in man has not been published before In the present study a significantly prolonged SRT was observed and this prolongation was probably related to the bradycardia induced by the β-receptor blockade. Verapamil is reported to prolong SRT (2) In this study however verapamil had no effect either on SRT or on the spontaneous atrial rate.

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MINOXIDIL—HAEMODYNAMIC AND CLINICAL EXPERIENCES WITH A NEW PERIPHERAL VASODILATOR

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Abstract Minoxidil, a new peripheral vasodilator given orally to hypertensive men in single doses of 5–25 mg, *produced no haemodynamic changes within one hour after* administration. After repeated oral doses within 24 hours to total of 15–45 mg and after 10 mg t.i.d. orally for one week, moderate decreases in BP were seen concomitant with tendencies to increased heart rate and cardiac output. Clinically oral minoxidil 10–30 mg daily in combination with diuretics and adrenergic β-receptor blocking agents achieved an improved BP control in five patients with sustained arterial hypertension and unsatisfactory response to previous treatment. However in four of the five patients minoxidil had to be withdrawn because of side-effects. It is concluded that minoxidil, producing hypotensive effect of slow onset may find place as a therapeutic addition to symptomatic patients with severe and therapy-resistant hypertensive cardiovascular disease provided adequate measures are taken to counteract side-effects especially water retention and development of oedemas.

The theoretically attractive approach of combining adrenergic β-receptor blocking agents with peripheral vasodilators for treatment of arterial hypertension has aroused new interest in the development of compounds with peripheral vasodilating properties (2 3 6 7 13 14 15)

One such compound is minoxidil a piperidinopyrimidine derivative, shown in animal experiments to possess a direct relaxant effect on vascular smooth muscle (1) In man minoxidil given orally in doses of 15–80 mg a day for one week causes haemodynamic changes characteristic of peripheral vasodilators, i.e. decreased BP concomitant with increases in heart rate and cardiac output (3) Preliminary clinical studies with minoxidil have yielded promising results in hypertensive patients usually in combination with adrenergic β-receptor blocking agents and diuretics (4 8 10)

The present investigation was undertaken to evaluate minoxidil further Five hypertensive men were studied haemodynamically before and after single and repeated doses of the drug. In a pilot study the clinical effects were observed in another five patients in whom previous therapy had been unsatisfactory

MATERIAL AND METHODS

Haemodynamic study

Eight male in-patients between the ages of 30 and 55 years *with benign essential hypertension gave their informed* consent to participate in the study On of them (no 11) had cardiac enlargement on X-ray (600 ml/m² BSA) with slight signs of pulmonary congestion, otherwise no patient had signs of cardiac or renal insufficiency Four patients were previously untreated. Propranolol had been given for short periods to three patients, but was withdrawn one week or more before the study The remaining patient had received total of 80 mg propranolol the day before the first study

In six patients the acute effects of single dose of minoxidil were studied, the systemic haemodynamics at rest in recumbency being determined before and one hour after an oral dose. Four patients in groups of two were given 5 and 10 mg, respectively and two patients received 15 and 25 mg, respectively of minoxidil administered in capsules containing 2.5 mg.

Without any other treatment in the meantime, five of these patients had their systemic haemodynamics in recumbency restudied one week later (in one case seven weeks later) after three oral doses of minoxidil within the preceding 24 hours, the last dose being given in the morning of the day of the study The same four patients as above in groups of two got total of 15 and 30 mg, respectively The patient who had previously received 15 mg in single dose was given *total of 45 mg.*

Two patients were studied at rest and during standardized bicycle ergometer exercise (750 kpm/min) in the sitting position before and after oral minoxidil 10 mg t.i.d for one week.

Table I *Clinical effects of minoxidil*

Auscultatory BP in the lying and standing (italics) positions, and HR in recumbency Averages of last three recordings before entering the study and of three measurements after 1 2 and 4 weeks on minoxidil

Alpr=alprenolol Chlor=chlorthalidone Clon=clonidine Furos=furosemide Guan=guanethidine HyChl=hydrochlorothiazide Hydr=hydralazine, Minox=minoxidil, Propr=propranolol

		Before minoxidil			During minoxidil				
Pat. no.	Age (y)	BP (mmHg)	HR (beats/min)	Treatment (mg/d.)	BP (mmHg)	HR (beats/min)	Treatment (mg/d.)	Duration of treatment (weeks)	Comments
1	55	182/127 *180/127*	63	Propr 480 Hydr 150 Chlor 50	196/120 *176/116*	116	Minox 40 Chlor 50–100 Alpr 1 800	5	Stopped minoxidil because of dyspnoea and dependent oedemas
2	46	232/124 *193/126*	56	Alpr 1 200 Hydr 200 Chlor 50 Clon 0 45	180/111 *176/113*	69	Minox 20 Chlor 50 Alpr 800 Clon 0.15 Furos 80	6	Stopped minoxidil because of dependent oedemas
3	57	206/118 *211/121*	82	Propr 480 Chlor 50 Guan 100	165/105* *177/107**	84*	Minox 10 Chlor 50 Alpr 800	3	Stopped minoxidil because of dyspnoea and angina pectoris
4	60	223/114 *218/115*	56	Propr 480 Hydr 150 HyChl 50	208/100 *158/102*	71	Minox 50 Chlor 50 Alpr 1 [illegible]00	8	Stopped minoxidil because of aggravated claudication
5	54	206/130 *204/137*	84	Propr 3[illegible]0 Chlor 50	160/102 *174/120*	87	Minox 10 Chlor 50 Alpr 800	18	No side-effects
Mean		210/123 *201/125*	68		182/108 *172/112*	85		8	

*Averages of tw measurements after 1 and 2 weeks on minoxidil.

The examination procedure for obtaining haemodynamic data and the techniques used for their analyses ha been described in detail elsewhere (12). Briefly the patients were brought to the laboratory in the morning afte overnight fast Under local anaesthesia polyethylene catheters were inserted into the brachial artery and an antecubital vein The venous catheter was advanced under fluoroscopy to the superior vena cava for subsequent central injections of dye The intra-arterial BP was registered on an Ultralette recorder by means of strain gauge (Statham P23Db) and the mean arterial BP was determined by electrical integration. The cardiac output ($\dot{Q}$) was determined using dye-dilution technique with bromsulphalein as the indicator and with intermittent sampling of arterial blood An ECG was continuously monitored, it was registered on the Ultralette recorder during the pressure recordings and during the $\dot{Q}$ procedure and used for calculations of the heart rate (HR). From the data for HR, mean brachial artery BP and $\dot{Q}$ the stroke volume (SV) and systemic vascular resistance (SVR) were calculated and expressed in ml/beat, and arbitrary units, respectively

After insertion of the catheters the patients rested for 30 min before the first measurements at rest were made with the patients in recumbency on the fluoroscopy table or comfortably sitting in an arm-chair $\dot{Q}$ during exercise was determined during the final minute of the exercise period, which lasted 10 min.

Clinical study

Five male out-patients 46–60 years of ge, were given minoxidil because of unsatisfactory response to other forms of antihypertensive treatment. Four of them had benign essential hypertension, one had benign sustained hypertension after reconstructive surgery for renal artery stenosis.

After withdrawal of previous treatment, minoxidil 5 mg orally b.i.d. was given together with alprenolol (Aptin®) and chlorthalidone (Hygroton®) The doses of minoxidil, alprenolol and chlorthalidone were then adjusted according to BP response and occurrence of side-effects especially tachycardia and dependent oedema, the highest dose of minoxidil given being 50 mg daily (Table I). The observation period aried from 3 to 18 weeks (average 8), during which the auscultatory BP was measured at regular intervals with mercury manometer Recordings were

Table II *Haemodynamic effects of minoxidil*

Individual findings in six patients at rest in recumbency before, one hour after a single oral dose and after repeated oral doses within 24 hours

Pat. no	Phase	HR (beats/min)	Brachial artery BP (mmHg) Systolic	Mean	Diastolic	$\dot{Q}$ (l/min)	SV (ml/beat)	SVR (U)
6	Before	72	186	125	84	8.7	121	14 4
	5 mg p.o	68	180	124	89	7 9	116	15 7
	15 mg p.o./24 h	72	158	109	80	7 4	103	14 7
7	Before	54	162	116	91	5 0	93	23.3
	5 mg p.o.	60	152	112	88	5.5	92	20.5
	15 mg p.o /24 h	66	131	105	81	6.3	96	16.7
8	Before	60	176	125	98	5.6	93	22.4
	10 mg p.o	62	173	123	96	5.6	90	22.0
	30 mg p .../24 h	72	161	122	94	7.6	106	16 0
9	Before	72	212	149	111	6.9	96	21 6
	10 mg p.o.	70	202	143	110	6.7	96	21 4
	30 mg p.o /24 h	76	205	144	111	7.8	103	18.5
10	Before	48	197	128	94	6 5	135	19 7
	15 mg p.o	48	181	124	91	7.5	156	16.6
	45 mg p. ./24 h	54	166	116	85	7.2	133	16 2
11	Before	70	237	168	130	6.7	96	25 1
	25 mg p.o.	70	231	164	122	7 2	103	22.8

obtained after 5 min of rest in recumbency and in the erect position after 1–2 min

RESULTS

Haemodynamic stud es

The haemodynamic findings are presented in Table II and Figs 1–3

No consistent changes indicative of drug effect, were seen one hour after administration of a single oral dose of 5–25 mg minoxidil (Fig. 1)

After repeated oral doses within 4 hours, all five patients had a lower brachial artery BP (−7 to −31 mmHg) concomitant with a tendency to increased HR and $\dot{Q}$ and the calculated SVR was lower in four of the patients (Fig 2) There was however no obvious dose–response relationship

The same tendencies were also seen in the two patients restudied after oral treatment with minox idil for one week but the changes observed were slight throughout (Fig. 3)

Clinical study

All five patients showed a decrease in BP as judged from the average of recordings after 1 2 and 4 weeks (Table I) but none became normotensive The average BP reduction in the recumbent position was 28/15 and in the erect position ?9/13 mmHg. The pulse rate at rest rose in every patient from an initial average of 68 to 85 beats/min.

The a erage weight increased from 85.5 kg during the previous treatment to an average of 89 7 kg after 1 2 and 4 weeks on minoxidil. In four patients the treatment had to be discontinued because of side effects in the form of oedemas increased symptoms of angina pectoris and claudication. Thus, in one patient (no. 2) chlorthalidone 50 mg daily did not prevent the development of dependent oedemas and furosemide had to be added. In another patient (no 1) sustained tachycardia was present in spite of continuous administration of alprenolol in ade quate doses. No abnormalities in routine blood liver and kidney tests were noted

DISCUSSION

Pharmacokinetic studies in hypertensive patient have shown minoxidil to be rapidly and completely absorbed after oral administration with maximal plasma concentrations occurring at on hour (5) Based on this, haemodynamically demonstrable effect was xpected to ppear in the present study one hour after a single oral dose. However no immediate cardiovascular effects were noticed even when the single dose of minoxidil had been in creased up to 25 mg, and only inconsist

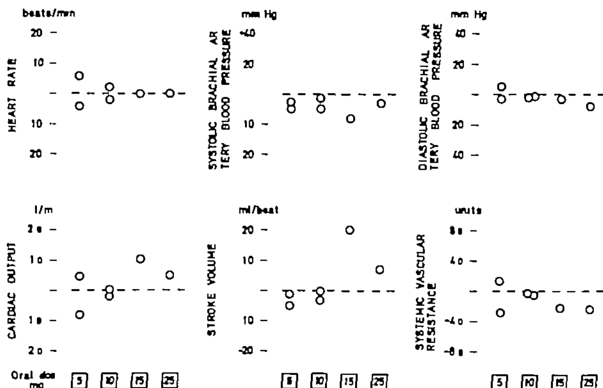


Fig 1 Haemodynamic responses to single doses of minoxidil 5–25 mg orally Hypertensive patients studied t rest in recumbency

non-decisive changes were observed after repeated doses within 24 hours or after treatment for one

indicates a slow onset of action of minoxidil ast to animal findings where a dose maximum hypotensive effect occurred after 4–6 hours and lasted for 24–48 hours after single oral doses (1) It is also contrary to the pharmacodynamic properties of other peripheral vasodilators such as hydralazines where the cardiovascular effects present themsel es as BP reduction with a compensatory increase in $\dot{Q}$ within 30 min after an oral dose of 100–150 mg (9)

A slow onset of action was also obvious in the small group of hypertensive patients followed clinically Animal studies have shown a specific retention of minoxidil in the femoral artery and aorta to occur after intraperitoneal application (11) and theoretically the slow onset of action could be due to a continued accumulation of minoxidil in the peripheral tissues particularly the essel walls.

In agreement with the results of others (4 8 10) the present pilot study confirmed minoxidil to be of therapeutic value in conjunction with other antihypertensive agents like diuretics and adrenergic β-receptor blocking agents for treatment of therapeutically difficult cases

The improved BP control was achieved at the expense of side-effects requiring withdrawal of minoxidil in four of five patients and this discouraged us from extending the present type of trial to cover a larger number of patients Concomitant therapy with saluretics and adrenergic β-receptor blocking agents was not enough to prevent development of oedemas in two patients and precipitation of angina pectoris in one patient One of the oedematous patients also reacted with persistent tachycardia in spite of a high dose of alprenolol Sodium retention has been demonstrated to be a consistent feature in patients treated with minoxidil and precipitation of myocardial ischaemia has also been described (3 10)

Side-effects of other kinds e g hypertrichosis have also been reported (4 10) and in addition minoxidil has in dogs been shown to produce necroses in the right atrium (1) Therefore it seems

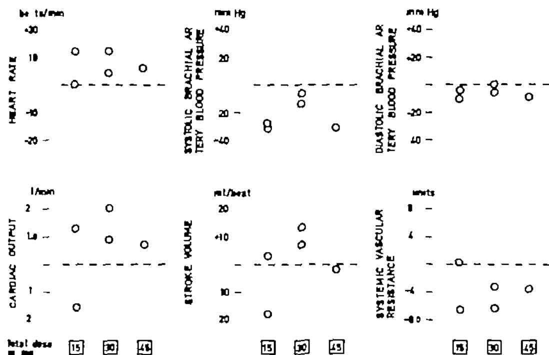


Fig 2 Haemodynamic changes in hypertensive patients given total of 15–45 mg minoxidil within 24 hours before the study

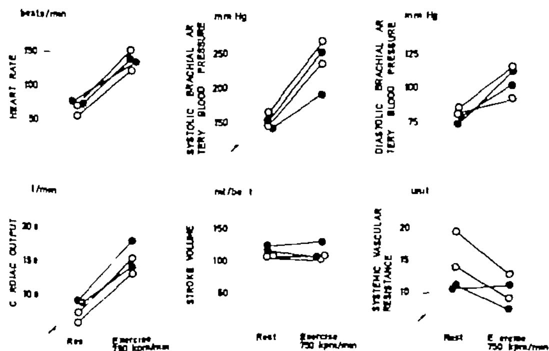


Fig 3 Systemic haemodynamics at rest and during exercise in the sitting position before (○) and after (●) oral treatment with minoxidil 10 mg t.i.d for one week in two hypertensive patients.

unlikely that minoxidil will become a drug suitable for routine treatment of initially symptom-free patients with benign arterial hypertension

Minoxidil may however as pointed out by several authors (3 8 10) be justified as a therapeutic addition to patients with very severe and drug-resistent arterial hypertensive disease. Adequate measures must then be taken to counteract side effects especially water retention and development of oedemas.

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THE EFFECT OF HYDROCHLOROTHIAZIDE AND AMILORIDE ADMINISTERED TOGETHER ON MUSCLE ELECTROLYTES IN NORMAL SUBJECTS

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Abstract Hydrochlorothiazide 150 mg daily and amiloride, 15 mg daily have been administered together to 10 normal subjects during one week. Muscle biopsies were performed before and after the period of administration of the diuretics and the material was analysed for water and electrolytes. The body weight decreased by 1 9 kg. The serum sodium, chloride and potassium concentrations decreased significantly and standard bicarbonate and blood pH increased. I muscle tissue extracellular water chloride and sodium contents and intracellular sodium concentration decreased but the muscle potassium content, the intracellular potassium concentration and the muscle magnesium content were unchanged A comparison with the results of an earlier study in which hydrochlorothiazide was given without amiloride showed that the intracellular potassium depletion and the increase in intracellular sodium concentration, observed with the benzothiazide diuretic could be fully prevented by simultaneous administration of amiloride. The degree of hypokalemia and alkalosis was also smaller with hydrochlorothiazide+amiloride than with hydrochlorothiazide alone.

Benzothiadiazine diuretics increase the excretion of potassium and thus may induce hypokalemia and intracellular loss of potassium This has been attributed to inhibition of sodium reabsorption in the distal tubulus proximal to the ion exchange site permitting a greater supply of sodium for utilization of the potassium—*sodium exchange mechanism (2)*

The diuretic agent amiloride acts at the distal renal tubulus where it interferes with the exchange of sodium for potassium (1) When given together with a benzothiadiazine diuretic, it further enhances the natriuretic action and prevents the potassium loss (15).

Bergström and Hultman (5) using a needle biopsy technique could demonstrate that administration of hydrochlorothiazide (150 mg daily) to normal subjects during one week resulted in a significant decrease *in muscle pota sium. The present study wa* undertaken in order to find out whether amiloride administered together with hydrochlorothiazide could prevent the expected intracellular pota sium loss induced by the thiazide

MATERIAL AND METHODS

Ten healthy volunteers, aged 24–35 were examined by means of needle biopsies taken from m quadriceps femoris before and after 7 days administration of hydrochlorothiazide (150 mg, 3×50 mg, daily) and amiloride (15 mg, 3×5 mg, daily). Venous blood wa collected from cubital vein at the time of biopsy care being taken to avoid errors due to stasping and handling of the samples (10)

Arterialized" capillary blood from finger tip was collected for determination of pH P_{CO_2} and standard bicarbonate. The body weight was recorded before and after the administration period of the diuretics The subjects who carried on their usual daily work, were not on a controlled diet, and the water and salt intake was not restricted.

The muscle biopsies were performed between 8 and 10 a.m. after an overnight fast.

The needle biopsy specimens were taken alternating from both legs before and after the period of diuretic administration. On each occasion sampling was done at two different sites: 1) 14–16 cm proximally to patella and 2) 20–22 cm proximally to patella. Each specimen which weighed 20–80 mg, was divided into 2–4 pieces, weighing 10–20 mg Visible fat and connective tissue were rapidly removed by dissection and the specimens were weighed on an electromagnetic balance. The specimens were dried at 90°C and weighed Neutral fat wa extracted with petroleum ether and the pieces were weighed again The water and fat contents were calculated The details of the biopsy technique, weighing procedure and fat extraction have been described earlier (3). Sodium, potassium and magnesium contents were determined by atomic absorption spectrophotometry and chloride was measured by electrometric titration (6) Serum lectrolytes and protein were analysed according to routine method (Autochemist) in the Clinical Laboratory separate samples of heparinized plasma were used for potassium and magnesium determination by emission and absorption flame spectrophoto-

Table I *The effect of hydrochlorothiazide (150 mg daily) and amiloride (15 mg daily) during 1 week on body weight and serum or plasma values in 10 normal subjects*

B = before A = after

Subj no.		Age (y)	Sex	B. wt. (kg)	Protein (g/l)	Blood pH	P_{CO_2} (mmHg)	Standard HCO_3^- (mEq/l)	Cl^- (mEq/l)	Na (mEq/l)	K (mEq/l)	Mg^{++} (mEq/l)
1	B	28	♂	64.2	77	7.41	40.5	24.8	104	143	4.3	1.5
	A			64.1	71	7.40	40.5	24.8	101	141	3.8	1.8
2	B	35	♂	87.0	80	7.41	38.5	24.5	103	143	4.1	–
	A			83.7	87	7.48	37.0	27.0	95	134	3.5	1.9
3	B	25	♂	72.8	71	7.42	41.5	26.0	104	144	4.5	–
	A			70.3	78	7.45	35.0	25.0	98	140	3.8	1.8
4	B	33	♀	62.2	78	7.40	37.0	23.0	106	144	4.0	1.5
	A			60.3	81	7.46	34.0	25.0	98	141	3.7	1.4
5	B	24	♀	55.4	74	7.49	34.0	27.0	103	142	4.6	1.6
	A			54.1	79	7.50	36.0	29.0	95	141	3.5	1.8
6	B	22	♂	74.9	78	7.40	35.0	23.0	104	143	4.0	1.8
	A			72.8	80	7.45	38.0	27.0	97	139	3.5	2.1
7	B	25	♂	69.5	73	7.42	39.0	25.0	101	142	4.7	1.5
	A			69.0	72	7.46	41.0	29.0	104	140	3.8	1.7
8	B	35	♂	78.5	69	7.43	41.0	27.0	106	141	3.9	1.6
	A			75.0	78	7.45	37.0	27.0	98	137	3.8	1.5
9	B	31	♀	58.0	74	7.43	30.0	22.0	106	144	4.5	1.6
	A			56.1	76	7.47	37.0	28.0	96	137	3.4	1.9
10	B	29	♀	47.3	72	7.45	35.0	25.0	108	144	3.7	1.5
	A			45.3	73	7.46	36.0	26.0	97	137	3.6	1.4

Table II *The effect of hydrochlorothiazide (150 mg daily) and amiloride (15 mg daily) during 1 week on muscle wate and electrolytes in 10 normal subjects*

B = before A = after *tot* = total, *e* = extracellular *i* = intracellular

		Per 100 g fat-free solids							Per l H_2O_i	
		H_2O_{tot} (ml)	H_2O_e (ml)	H_2O_i (ml)	Cl^- (mEq)	Na (mEq)	K (mEq)	Mg^{++} (mEq)	Na_i (mEq)	K_i (mEq)
1	B	343	34.6	308	5.38	7.83	46.35	8.96	10.417	149.3
	A	336	27.7	308	4.45	6.85	46.42	9.04	9.596	150.9
2	B	317	28.7	290	4.61	8.20	44.70	9.25	14.154	154.5
	A	331	29.2	301	4.37	7.26	45.19	9.12	11.125	150.4
3	B	325	30.8	294	5.20	7.90	47.02	9.06	10.596	159.6
	A	332	24.7	307	4.02	6.19	47.11	9.44	8.965	154.4
4	B	341	44.4	297	6.64	10.61	46.41	8.91	14.[illegible]46	156.5
	A	350	34.7	314	5.16	8.85	46.34	9.23	11.660	149.4
5	B	352	43.6	311	6.40	10.13	47.79	9.01	12.742	154.0
	A	355	36.1	318	5.16	8.56	47.80	9.06	11.750	151.1
6	B	331	25.4	305	4.33	6.96	46.67	9.01	10.928	153.2
	A	319	26.4	293	4.11	7.38	46.37	8.92	12.626	158.9
7	B	333	43.2	288	6.13	9.14	46.72	9.00	13.014	162.2
	A	325	35.4	290	5.40	7.63	47.91	9.39	8.422	165.5
8	B	339	52.1	287	7.46	10.47	46.41	8.74	12.290	161.8
	A	333	42.9	291	5.94	8.17	46.85	8.64	10.103	161.7
9	B	355	56.6	300	8.07	11.48	47.59	8.70	11.132	159.2
	A	328	32.2	293	4.55	8.10	45.92	8.67	12.670	157.1
10	B	330	39.1	291	6.07	10.52	47.33	8.82	16.889	162.7
	A	329	31.4	299	4.64	8.24	47.47	8.94	13.328	159.2

Table III *Body weight serum or plasma values muscle water and electrolytes before and after hydrochlorothiazide (150 mg daily) and amiloride (15 mg daily) administration during 1 week in 10 normal subjects*

Abbreviations as in Table II

	Mean		Differ ence	t	Sig-nifi-cance
	B	A			
B wt. (kg)	67.0	65 1	−1.9	5 62	
Serum or plasma values					
Protein (g/l)	74	78	+4	3 09	
Blood pH	7 43	7.46	+0 03	4 17	**
P_{CO_2} (mmHg)	37.2	37.2	0	–	–
Standard HCO_3^- (mEq/l)	24 7	26.8	+2.1	2.99	
Cl^- (mEq/l)	105	98	−7	5.20	
Na (mEq/l)	143	139	−4	5.26	**
K (mEq/l)	4.2	3.6	−0.6	5 09	***
Mg^{++} (mEq/l)	1 6	1 7	+0 1	1.87	–
Muscle water and electrolytes					
Per 100 g fat-free solids					
H_2O_{tot} (ml)	336	334	−2	0 74	–
H_2O_e (ml)	39 9	32.1	−7.8	3.55	**
H_2O_i (ml)	297	301	+4	1.52	–
Cl^- (mEq)	6 0	4.8	−1.2	4.25	**
Na (mEq)	9.3	7 7	−1.6	5 05	**
K (mEq)	46.7	46.7	0	0 02	–
Mg^{++} (mEq)	9 0	9 1	+0 1	1.59	–
Per l H_2O_i					
Na_i (mEq)	12.6	11.0	−1.6	2.48	
K_i (mEq)	157	156	−1	1 14	

* 0.01<p<0.05 ** 0.001<p<0 01 *** p<0.001

metry respectively Standard bicarbonate, pH and P_{CO_2} were determined in whole blood by the Astrup micro-method (13) Tissue water and electrolyte contents were referred to *100 g fat-free solids.*

The determination of extra- and intracellular water was based on the chloride method Chloride is freely diffusible across the skeletal muscle fibre membrane and is distributed according to Nernst s equation (8). Taking the resting membrane potential of muscle in normal man to be 87.2 mV (7), the Cl_e/Cl_i ratio calculated from Nernst equation will be 26/1 If the total water and chloride content of the muscle tissue and the extracellular chloride concentration (obtained by correcting the plasma chloride concentration for a Donnan factor and a factor for plasma water (3)) are known, extra- and intracellular water volumes and intracellular electrolyte concentrations can be calculated (4 9).

RESULTS

General condition

Three subjects complained of slight to moderate fatigue and one had leg cramps. Three subjects experienced increased appetite and two increased thirst. No severe side-effects were observed

Body weight serum electrolytes and protein and acid–base data (Tables I II and III)

The body weight decreased A slight fall in sodium concentration and a more pronounced fall in chloride concentration were observed The potassium concentration decreased by 0 6 mEq/l which was highly significant. An increase in standard bicarbonate and blood pH also occurred

Muscle water and electrolytes

Significant decreases in extracellular water chloride and sodium contents occurred. The muscle potassium content and the intracellular potassium concentration were not changed but the intracellular sodium concentration decreased slightly The muscle magnesium content was unchanged

Comparison between hydrochlorothiazide + amiloride and hydrochlorothiazide without amiloride (Table IV)

The present results were compared with the results of a similar study presented earlier in which ten normal subjects were given 150 mg hydrochlorothiazide daily during one week (5).

The mean decrease in body weight was exactly the same (1 9 kg) with both regimens indicating that the degree of dehydration induced was similar The fall in extracellular potassium concentration and the degree of metabolic alkalosis (rise in standard bicarbonate) was greater with hydrochlorothiazide than with hydrochlorothiazide+amiloride. Hydrochlorothiazide administration resulted in a significant fall in muscle potassium and intracellular potassium concentration, and a significant increase in intracellular sodium concentration, whereas hydrochlorothiazide+amiloride had no effect on muscle potassium but induced a slight decrease in intracellular sodium concentration i.e an effect which was opposite to that of hydrochlorothiazide alone

According to our records hydrochlorothiazide alone appeared to induce more pronounced side effects than hydrochlorothiazide+amiloride (see "General condition"). Thus with hydrochlorothiazide no less than six subjects complained of severe fatigue vertigo and nausea and two suffered from constipation Increased thirst was experienced

Table IV *Comparison between the effect of hydrochlorothiazide and hydrochlorothiazide + amiloride during one week in 10 normal subjects*

	Plasma (mEq/l)			Muscle Fat-free solids (mEq/100 g)	Muscle Intracellular water (mEq/l)	
	ΔNa	ΔK	ΔHCO_3	ΔK	ΔNa^+	ΔK
A Hydrochlorothiazide (150 mg/d.)	−0.9	−1 11	+3 6	−2.7	+4.8	−12.5
B Hydrochlorothiazide (150 mg/d.) + amiloride (15 mg/d.)	−4.3	−0.60	+2.1	−0 06	−1.6	−1 4
A−*B* (*t*)	3 16	3.83	1.55	3 12	5.31	3 91
Significance	**	**	–	**		**

0.001<p<0.01 p<0.001

by seven subjects It is conceivable that mild potassium depletion was a factor of importance for the development of these side-effects

DISCUSSION

In the present investigation the hydrochlorothiazide dose (150 mg daily) was larger than usually recommended for maintenance therapy of patients with cardiac insufficiency or hypertension The proportion between the amiloride and hydrochlorothiazide doses was 1 10 i e the relation currently used in a fixed drug combination (Moduretic®) on the market Our results demonstrate that by combining amiloride with hydrochlorothiazide it is possible completely to prevent the intracellular potassium loss which oc in normal subjects after administration of lorothiazide alone

However amiloride could not fully prevent the development of hypokalemia and metabolic alkalosis induced by the thiazide diuretic although the fall in plasma potassium concentration was less marked with hydrochlorothiazide+amiloride than with hydrochlorothiazide alone

Another effect of a thiazide diuretic namely an increase in the intracellular sodium concentration (5) could also be prevented by combining the thiazide with amiloride Since the increase in intracellular sodium after thiazide administration probably occurs in exchange for potassium lost from the cells our results further support the hypothesis that administration of amiloride perserves the intracellular electrolyte milieu during thiazide administration The slight fall in extracellular potassium concentration is most probably a consequence of the co-existing slight metabolic alkalosis (1? 14)

Intracellular potassium depletion, with consequent increase in intracellular sodium concentration is known to increase the sensitivity to digitalis thus leading to increased risk of digitalis intoxication (11) Such intoxications are observed with increasing frequency due to the widespread use of diuretics which increase the excretion of potassium. Since amiloride is able to prevent the thiazide-induced fall in intracellular potassium concentration as well as the increase in intracellular sodium concentration it appears to be a suitable drug for patients in whom the risk of digitalis intoxication must be considered Also in other conditions where a patient is at risk of diuretic-induced potassium depletion amiloride should be considered as an alternative to oral potassium supplementation.

Our results indicate that there is no risk of hyperkalemia when amiloride is combined with hydrochlorothiazide in the proportions used in this study at least in subjects with normal renal function.

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TURNOVER OF PLASMA CHOLESTEROL *IN PATIENTS WITH CHOLESTEROL GALLSTONES*

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Abstract Following the i. injection of about 30 µCi of 1-α-2-α-³H-cholesterol the specific activity decay of plasma cholesterol has been analysed in terms of a 2-pool model in eight patients with radiolucent gallstones and two healthy controls. In each subject some kinetic parameters were calculated, including the input into and the size of the rapidly exchangeable cholesterol pool. The results were compared with those in 16 controls from another study Statistical analysis failed to demonstrate significant differences in any of the parameters between the gallstone patients and the combined controls indicating that patients with cholesterol gallstones have normal input into and size of the rapidly exchangeable cholesterol mass. This conclusion is discussed in relation to similar studies, and it is speculated that gallstone patients may have decreased absorption of exogenous cholesterol.

In recent years the physicochemical basis of cholesterol gallstone formation has been studied intensively It seems to be well established that the disease occurs in association with bile that contains an excess of cholesterol in relation to its content of bile acids and lecithin (1) and that the bile is lithogenic before it enters the gallbladder (16 19). Such an abnormality could arise from an increase in hepatic secretion of cholesterol from a decreased output of bile acids and/or lecithin or from a combination of these changes. So far most studies tend to show that a lithogenic bile is due preferably to a deficiency of bile acids relative to cholesterol (? 17 18).

Lately however Grundy et al (7 8) have emphasized that concomitantly an increased cholesterol secretion may play an important role in the production of lithogenic bile This observation was based upon direct measurements of duodenal biliary lipids in American Indians and the authors think that their results are consistent with an increase in cholesterol synthesis.

In order to further explore this aspect we have estimated some kinetic parameters, including the size of the rapidly miscible pool and the input into this pool in a number of Caucasian patients with radiolucent gallstones.

MATERIAL AND METHODS

Eight patients with radiolucent gallstones in well functioning gallbladders, and two healthy control were studied (Table I). The examinations were performed on an outpatient basis in the course of about 10 weeks N special diets were given and the weights of the patients remained unchanged Any medical treatment for other diseases was maintained, but no drugs known to affect liver function or lipid metabolism were given. The liver function tests (serum alanine aminotransferase prothrombin time, alkaline phosphatase, serum protein electrophoresis and serum bilirubin) were normal in all patients and controls. None of the patients or controls had symptoms or signs of obstructive biliary disease.

Experimental procedure 1-α-2-α-³H-cholesterol was purchased from The Radiochemical Centre Amersham, in benzene solution This was dried under N and the tracer redissolved in ethanol Purity exceeded 95% according to thin-layer chromatography One ml of the ethanol solution was sterilized by passage through Millipore filter and added to 9 ml of Intralipid® (Vitrum) under sterile precautions. The specific activity was measured by liquid scintillation counting (Packard Tri Carb), and 2–3 ml injected to the patients and controls The radioactivity dose was some 30 µCi In three of the patients the disappearance from the plasma was followed during the first 15 m which demonstrated that the radiocholesterol was cleared from the circulation with half-life of approximately 10 min. I the same patient the distribution of radioactivity in lipoproteins was estimated by lipoprotein electrophoresis followed by localization of radioactivity on the strip In every case the distribution conformed to that of cholesterol in healthy controls (4)

Table I *Clinical data for eight patients with radiolucent gallstones and two controls*
MW = molecular weight, R = limits of control values of the laboratory

Case no.	Age (y)	Sex	Height (cm)	Weight (kg)	% of ideal weight	Plasma cholesterol (mg/100 ml) MW 387 R 140–300	Plasma triglycerides (mg/100 ml) MW 885 R 35–160	History
Patients								
1	73	♀	160	80	136	263	150	Myocardial infarction
2	64	♀	161	85	142	283	?	Cerebral thrombosis
3	63	♀	165	80	129	263	239	
4	62	♂	171	81	119	263	124	Gastric resection
5	73	♀	155	75	134	383	204	Myocardial infarction
6	48	♂	167	68	105	279	124	
7	72	♀	153	82	152	194	106	Cerebral thrombosis
8	45	♀	170	60	91	232	168	
Controls								
1	43	♂	182	78	104	286	?	
2	37	♂	180	71	96	309	?	

Fasting plasma samples were obtained first twice, later once weekly from the patients in the course of the first 10 weeks after injection of the tracer. Plasma cholesterol was determined according to Abell (10). Plasma triglycerides were analysed by the Boehringer Mannheim UV test. Radioactivity was measured on the hexane extract by liquid scintillation counting. All analyses were carried out in duplicate.

Calculations The results were analysed as described by Goodman and Noble (5) in terms of a 2-pool model (Fig. 1). The specific radioactivity of plasma cholesterol was plotted in semilogarithmic system against time. In all cases the plots conformed well to a 2-pool model, with a curved part during the first 4–5 weeks, and a linear plot ond this time (Fig 2) The two lines used for the ution of the curve int two exponentials were drawn, the kinetic parameters were calculated according to Goodman and Noble (5) By assuming that the removal rate of cholesterol from pool B to outside the system (k_B) approximates zero it was possible to calculate the rate constants for transfer between the two compartments (k_{AB} and k_{BA}), and for removal of cholesterol from pool A to outside the system (k_A). This assumption has been evaluated by several authors (5 6, 20) and is generally accepted

The results were subjected to statistical analysis by means of Mann-Whitney s rank sum test (21).

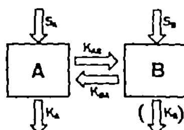


Fig 1 Model for kinetics in 2-compartment system (9). A = the rapidly exchangeable pool A. B = the slowly exchangeable pool B S_A S_B = rate of entry of cholesterol into pools A and B. K_A K_B = rate constants for outputs from pools A and B K_{AB} K_{BA} = rate constants for transfer between the two compartments. The parentheses indicate the assumption, that output from pool B takes place only through pool A.

RESULTS

The parameters of cholesterol kinetics are given in Table II and Fig. 3 In view of the few controls in our study the results were compared with those from Sandhofer et al (15) who used essentially the same method

The statistical analysis failed to demonstrate significant differences ($p = 0.1$) between any of the parameters from the gallstone patients and the compiled controls

DISCUSSION AND CONCLUSION

Some limitations of the 2-pool method have to be considered before making comparisons with other results and drawing final conclusions

Firstly it has been shown by long-term studies that the cholesterol decay curve can be better de-

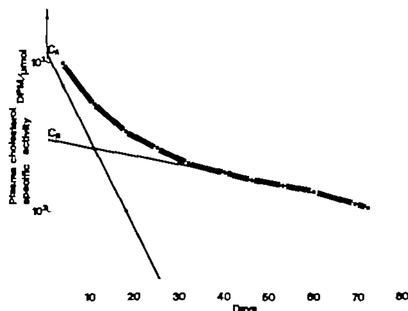


Fig. 2. Decay curve of the specific activity of plasma cholesterol in patient 6. The straight lines illustrate the resolution of the curve into two exponentials.

Table II. *Kinetic data of plasma choleste ol turnover in eight patients with radiolucent gallstones two controls and 16 controls from anothe study*

α = fractional turnover rate of the rapidly miscible pool A, β = fractional turnover rate of the slowly miscable pool B. k_{AA} = rate constant for the total removal from pool A. k_{BB} = rate constant for the total removal from pool B. M = size of pool A. PR_A = rate of input into pool A. k_{BA} = rate constant for transfer from pool B to pool A. k = rate constant for removal from pool A excluding transfer to pool B (exit of cholesterol from pool A to outside the system). k_{AB} = rate constant for transfer from pool A to pool B

Case no.	α day⁻¹	β day⁻¹	k_{AA} day	k_{BB} day⁻¹	M (mg/kg)	PR_A (mg/day/kg)	k_{BA} day⁻¹	k day⁻¹	k_{AB} day⁻¹
Patients									
1	0.1284	0.0141	−0.1041	−0.0384	330	15.5	0.0384	0.0471	0.0570
2	0.1650	0.0141	−0.1275	−0.0516	279	12.6	0.0516	0.0451	0.0824
3	0.1540	0.0154	−0.1203	−0.0491	340	16.4	0.0491	0.0483	0.0720
4	0.1386	0.0164	−0.1010	−0.0540	369	15.6	0.0540	0.0421	0.0589
5	0.1650	0.0164	−0.1348	−0.0466	267	15.5	0.0466	0.0581	0.0767
6	0.1444	0.0139	−0.1155	−0.0428	397	18.6	0.0428	0.0468	0.0687
7	0.1540	0.0141	−0.1305	−0.0376	273	15.7	0.0376	0.0578	0.0727
8	0.1540	0.0169	−0.1212	−0.0497	368	19.3	0.0497	0.0524	0.0688
Mean	0.1504	0.0152	−0.1194	−0.046	328	16.2	0.0462	0.0497	0.0697
S.E.M.	0.0045	0.0005	0.0043	0.0022	18	0.7	0.0022	0.0021	0.0030
Controls									
1	0.1824	0.0175	−0.1261	−0.0738	374	16.2	0.0738	0.0433	0.0848
2	0.1444	0.0163	−0.1144	−0.0463	369	18.7	0.0463	0.0508	0.0636
16 controls from Sandhofer et al. (15)									
Mean	0.1606	0.0162	−0.1308	−0.0460	326	18.3	0.0460	0.0568	0.0740
Mean of all controls	0.1609	0.0163	−0.1296	−0.0476	331	18.2	0.0476	0.0557	0.0740
S.E.M.	0.0044	0.0008	0.0041	0.0018	8	1.0	0.0018	0.0033	0.0031
P (patients and all controls)[b]	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1

Calculated by assuming that $k_B = 0$
Mann-Whitney rank sum test.

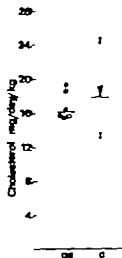


Fig 3 Input of cholesterol into pool A in eight patients with radiolucent gallstones (*gs*) and two controls from the present study plus 16 controls from Sandhofer et al. (15) (*c*) O=results from the present study

scribed by a 3-pool model (6 14) For all practical purposes estimations of the size of pool A are the same with both methods but if the third pool is neglected the input into the rapidly exchangeable pool A will be overestimated by about 8–9% and the size of the slowly exchangeable pool severely underestimated For this reason we have omitted an estimation of this compartment.

ondly Nestel et al (*12*) *found that compart* analysis tends to overestimate the cholesterol tion rate by about 10% in obese individuals compared to the results achieved by means of the sterol balance technique Since we have used the 2-pool model and since most of our patients were obese these considerations imply that our results for input into pool A represent maximum values whereas our estimations of the size of this pool should be valid

The size of pool A (M_A) in our patients (328±18 mg/kg) was essentially the same as that in the controls (331±8 mg/kg). Using the 3-pool model Hoffman et al (11) found the size of pool A to be 390±20 mg/kg in eight gallstone patients and were *not able to demonstrate* differences from results achieved by other authors in patients with various hyperlipidaemias Consequently it is reasonable to assume that the amount of rapidly exchangeable cholesterol is within normal limits in patients with cholesterol gallstones This is of particular interest since observations indicate that biliary cholesterol and de novo synthesized bile acids are derived primarily from this pool (13 20) Thus it is unlikely that the diminished bile acid pool in patients with cholesterol gallstones (17) can be due to a diminished amount of readily available cholesterol

The input into pool A (PR) was 16.2±0 7 mg/day/kg in the gallstone patients versus 18.2±1 0 mg/day/kg in the controls, the difference being non-significant. To our knowledge only one other group has performed a similar study (11). Using the 3-pool method the input into pool A was found to be 16.2±0 08 mg/day/kg in eight gallstone patients and no differences were observed from controls collected from the literature Taken together these results indicate that patients with cholesterol gallstones have normal rates of input of cholesterol into the rapidly exchangeable cholesterol mass

Grundy et al (7 8) using the sterol balance technique, found indications of increased synthesis of cholesterol in American Indian women with gallstones (17 7 mg/day/kg) compared to Indian men without stones (14.3 mg/day/kg). This result might seem to conflict with ours but it must be stressed that the synthesis of cholesterol is not the same as the input into pool A The latter parameter comprises both the endogenous synthesis and the absorbed amount of exogenous cholesterol (5 9). Thus the results of Grundy et al and our study can be combined in a non-conflicting way if one assumes that the absorption of cholesterol is diminished in gallstone patients This speculation conforms well to the facts that the absorption of cholesterol is highly dependent upon the presence of bile acids in the small intestine (3) and that the biliary secretion of bile acids is decreased in gallstone patients (7 8)

Although this indirect suggestion needs confirmation it does afford the possibility of a more detailed concept of the genesis of gallstone including the recent emphasis placed by Grundy et al (7 8) upon the role of cholesterol

ACKNOWLEDGEMENTS

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ANOREXIA NERVOSA AND THE FREQUENCY OF SEX CHROMATIN POSITIVE CELLS

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Abstract Five consecutive cases of anorexia nervosa in women have been investigated for variation in the frequency of *sex chromatin-positive cells*. The results showed a statistically significant relation between body weight and the frequency of sex chromatin-positive cells the amount of sex chromatin increasing with rising body weight. These findings are difficult to explai but the results might conceivably be used at least in some cases as an index of changing basal biological functions in anorexia nervosa.

In recent years papers have been published on the relation between endocrine disorders in women and low frequency of sex chromatin-positive cells. Relations of this type have been recorded both in congenital adrenal hyperplasia (4 13) and in hypothyroidism (7) It is also known that the frequency of sex chromatin-positive cells tends to decline n relation to a high content of oestrogenic hormone in the blood and that administration of other steroid hormones and ACTH can reduce the frequency of sex chromatin (14 16) Further t has been recorded that during the first weeks of infancy the frequency of sex chromatin is low rising thereafter to the levels found in adult women (15 16)

During the last ten years a number of reports have appeared concerning the coincidence of anorexia nervosa and Turner s syndrome (karyotype 45 X) in the same woman Seven such cases have been published (2 3 5 8 9 10, 12) and although it is not yet clear to what extent they represent anything more than random coincidence the coexistence of two such uncommon disorders is certainly worthy of note It has been suggested that in all cases of anorexia nervosa, complete karyotype analysis advisable in order not to miss cases of mosaicism

While a combined case of this type was under study some years ago it was observed in the hospital genetic laboratory that a girl with anorexia nervosa showed a remarkably low frequency of sex chromatin-positive cells during the anorectic phases of her illness On suspicion of Turner's syndrome (mosaicism) karyotype analysis was carried out but this proved to be normal Repeated determinations of sex chromatin revealed a striking rise in conjunction with increased body weight (1) This observation stimulated closer study of the changes in sex chromatin in anorexia nervosa.

MATERIAL AND METHODS

The investigation was carried out upon five consecutive female cases of anorexia nervosa referred to our clinic Their ages varied from 17 to 34 (mean 27 8). The diagnosis was *established according to the following criteria.* Anorectic behaviour as defined by Palazolli Selvini (11), i.e. "the conscious and stubborn determination to emaciate oneself despite the presence of an intense interest in food Additional requirements were high degree of progressive weight loss, secondary amenorrhoea and other signs of secondary hypogonadism laboratory evidence of reduced thyroid function without clinical sign of myxoedema and normal distribution of axillary and pubic hair

On admission to hospital each patient had buccal smear which was examined for the presence of sex chromatin Th buccal film wa fixed in alcohol and stained with tannin and 400 cell were assessed in every specimen Repeat test were made every second week during the hospital stay Simultaneously with each test, all the patients had estimation of total oestrogen in the urine and some of them also had determination of the thyroid functional lev l and the content of gonadotropins (FSH and LH) in the blood Treatment for all the patients was standardized and consisted of bed rest for the first two or three weeks, administration of small doses of

Table I *Frequency of sex chromatin-positive cells (%) related to current body weight (kg)*

	Case 1		Case 2		Case 3		Case 4		Case 5	
No of observations	Sex chromatin	B wt.	Sex chromatin	B wt.	Sex chromatin	B wt.	Sex chromatin	B wt.	Sex chromatin	B wt.
1	20	38.9	23	41 7	15	30.4	20	68.5	20	36 4
2	24	40.9	26	44 1	33	34 9	19	67.5	22	38.5
3	29	42.7	32	45.5	31	36.0	22	68.6	29	39 1
4	25	45.5	33	46.2	28	39 1	24	73 0	28	41.2
5	29	46.4			33	39.0	29	73.3	28	43 4
6	39[a]	54.6[a]			34	39.5	35	73.9	33	44.0
7					28	40.5	28	75.0	31	42.1
8					30	43.5	32	75.5	32	42.8
9									32	40 4
10									29	42.0
11									34	41.9
	0 93		0.96		0.61		0.87		0.78	
p	<0.01		<0.05		N.S.		<0.01		<0.01	

Recorded 5 months after the 5th observation.

chlorpromazine (max. 75 mg daily) and carefully graduated increase of caloric intake. At first this was mainly in the form of fluid nutrients, which were subsequently progressively replaced by normal diet. The investigations were discontinued when the patients had increased so much in weight and were in such physical and mental condition that they were considered fit for discharge or when they requested this. At the time of discharge none of the patients had had return of menstrual periods.

RESULTS

Table I shows the frequency of sex chromatin-positive cells in the five patients as determined in successive tests set in relation to body weight time of the tests The number of observations d from a maximum of 11 to a minimum of 4 bservations per patient (mean 7 4) It is clear from the Table that in every case there was a strong correlation between body weight and the frequency of sex chromatin, the amount of the latter rising with increasing weight The correlation coefficients varied between 0 61 and 0.93 Testing by means of Fischer s -transformation showed that all but one of the figures were statistically significant. The mean value of the correlation coefficients was similarly determined by means of Fischer s *z* transformation and amounted to 0.82 ($p<0\ 001$). Homogeneity testing by means of χ^2-analysis carried out on the transformed correlation coefficients showed no significant deviation of any of the recorded figures from the rest.

Table I does not include data from the hormonal analyses In general these showed no correlation either with body weight or with the frequency of sex chromatin, they did not vary significantly during the investigation period and above all showed no definite increasing tendency Only in one case (no 4) was it noted that as body weight increased the oestrogen output in the urine became normal It should be mentioned that the weight of this patient on admission was as much as 68.5 kg. However this was about 10–12 kg below her normal weight and in other respects her condition was one of undoubted anorexia nervosa.

DISCUSSION

In my material the amount of sex chromatin varied with the body weight a rise in weight being associated with a statistically significant increase in the frequency of sex chromatin. The frequency of sex chromatin-positive cells varied from a minimum of 15% to a maximum of 39% Most of these figures lie within the normal range of variation for sex chromatin frequency as reported by Smith et al (15) (19–49%) and by Hamerton (6) (36–76%).

The findings of this study are difficult to interpret. It has already been mentioned that the frequency of sex chromatin is influenced by hormonal factors It has also been observed that extensive burns cause a drastic fall of sex chromatin during the acute stage of the injury (17) In anorexia nervo-

sa, reduced levels of oestrogenic hormones are found in the blood and urine, as well as low levels of gonadotropic hormones (FSH and LH) Previous *observations indicate that high oestrogen content in* the blood correlates with low sex chromatin fre quency and my findings therefore cannot be ex plained on these grounds. It has also been shown that administration of adrenal cortical steroids or ACTH similarly induces a fall in the frequency of sex chromatin. In anorexia nervosa however there is a low urinary output of corticosteroids and the levels of plasma cortisols are normal There is therefore no parallel in this respect either On the other hand in anorexia nervosa there is a reduction of metabolism as measured *for example by the BMR* or other tests of thyroid function. As mentioned above low frequency of sex chromatin is found in hypothyroidism and this finding is therefore in line with my observations.

Weight gain is not the only sign of improvement in a patient with anorexia nervosa There is a rise in such *biological variables as basal temperature*, pulse rate and blood pressure and these often pres- age recovery before there is any change in weight. The present investigation suggests that change in the frequency of sex chromatin-positive cells is another such variable and that at least in some cases it can serve as an index of improvement in basal biological functions.

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Announcements

The Pio Instituto di S Spirito ed Ospedali Riuniti Roma in collaboration with the Accademia Lancisiana di Roma announces a competition for the 1975 G M *Lancisi International Prize for Medical Science* of Lit. 10 000 000 to be assigned to the author(s) of an original unpublished scientific work dealing with the subject *Clinical Immunology*

Those intending to enter for the prize should send the following to Premio Internazionale Scientifico G M Lancisi presso la Presidenza del Pio Instituto S Spirito – Borgo S Spirito 3 I-00193 Roma Italy by Dec 31 1975

1) Application on plain paper to participate in the competition) Nine copies of the work entered for the prize in Italian or in English, each copy bearing the signature of the author(s) All works accompanied by positive copies of any illustrations should include a synopsis in Italian or English of not more than 1 000 words as well as a full bibliography of the subject dealt with. 3) Certificates testifying to the birth recidence and citizenship of the competitor(s) 4) A copy of the curriculum vitae and of the scientific activity of the competitor(s) with a list of published works 5) A declaration signed by the author(s) testifying to the original nature of the work the institute or other place where the work has been carried out that said work is unpublished and that it has not been submitted and will not be submitted until such time as the G M Lancisi prize been assigned to other prize competitions.

1

International Heparin Symposium will be held in London July 18–19 1975

Further information Mr V V Kakkar Department of Surgery King s College Hospital Denmark Hill London SE5 8RX England

The XII Congress of the European Dialysis and Transplant Association (E.D T.A) will be held at the Copenhagen Bella-Center Aug. 26–29 1975 The official languages of the congress are English and French

Further information E D T.A. Congress DIS-Congress Service Knabrostraede 3 DK 1210 Copenhagen K Denmark.

International Coloquy on Lipoproteins and Hyperlipidemias will be held in Lisbon, Portugal Sept. 3–6 1975

Further information. Prof M J Halpern, Dept. of Biocemistry Faculty of Medicine of Campo Santana, Lisbon Portugal

2nd International Meeting of Medical Advisers in the Pharmaceutical Industry will be hald in Palazzo dei Congressi Florence Italy Oct. 13–15 1975

Further information Organizing Committee Secretariat Dr N Bergamini Dr V Bachini Via R. Lepetit 8 Milano Italy

Acta med. scand Vol 197 pp 431–432, 1975

TOTAL DISAPPEARANCE OF A FATAL PULMONARY EMBOLUS DURING STREPTOKINASE THERAPY OF AN ILIOFEMORAL THROMBOSIS

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Abstract During streptokinase treatment of an iliofemoral thrombosis, a formerly healthy man developed clinical signs of major pulmonary embolism. I spite of all attempts at resuscitation the patient died after 1.5 hours. The autopsy showed no signs of remaining thrombus or emboli. The probable explanation is that the thrombu had partially been lysed and that the remaining parts formed an embolus large enough to cause considerable haemodynamic changes which could not be influenced. Lysis continued pre and postmortally and was complete at the time of autopsy

For many years the relationships between clinical and pathological findings have been well established Most often the findings at autopsy can be predicted and sometimes the clinical picture is so characteristic that an autopsy seems almost unnecessary However therapy changes and it should not be forgotten that the introduction of new methods may completely change a morphological picture familiar to a pathologist Having met with such a situation in the unsuccessful treatment of a man with a deep enous thrombosis who died from a classical pulmonary embolism, we found it of particular interest to report the features of this case.

CASE REPORT

A formerly healthy and quite alert 77-year-old man was hospitalized because of pain and swelling of the right leg for two days. The obvious clinical diagnosis was an iliofemoral thrombosis. Ascending phlebography showed an almost total occlusion of the veins up to the inferior vena cava Apart from peripheral thrombocyte count of 56000 and a bleeding time of 12 min there were no abnormalities on laboratory examination at this time Active thrombolytic treatment was started by constant infusion of streptokinase (Kabikinase® Kabi, Stockholm Sweden) N initial dose titratio was performed Instead the patient received 300000 IU streptokinase during one hour after which the treatment continued with 100000 IU/hour

Four hours after treatment started the thrombin time was more than double the normal indicating a fibrinolytic effect After 15 hours treatment the patient was definitely improved. He had no pain and only moderate swelling of the leg remained No complications to the thrombolytic therapy had been observed. Two hours later the patient suddenly deteriorated with all the classical signs of a major pulmonary embolism, including circulatory collapse.

The patient was immediately treated with oxygen and assisted ventilation and was rapidly transferred to the Intensive Care Unit. Further he received papaverine (80 mg) meperidine (50 mg), theophyllamine (200 mg) i.v and isoprenaline (1 mg) i 500 ml of 5.5% dextrose solution. Nevertheless he did not improve and because of cyanosis he was intubated and ventilated with pure oxygen and positive endexpiratory pressure of 10 cm H_2O by means of an Engström ventilator 300 (LKB-medical, Stockholm, Sweden) without any evident improvement.

During this treatment the ECG showed gradually increasing signs of myocardial hypoxia, but no arrhythmia was observed. The ystolic BP varied between 50 and 80 mmHg. After one hour treatment the circulation failed completely though the ECG showed fairly regular rhythm. N pulses could be felt and the pupils became dilated. External cardiac compression was without effect and at no time could any sufficient circulation be obtained. The resuscitation was stopped after 1.5 hours.

Four hours after death the body was transferred to a cold room and the autopsy was performed 96 hours later At this time neither pulmonary embolus nor deep venous thrombus could be found The heart was dilated but not hypertrophic and there were signs of venous congestion in both lungs and the liver No signs of myocardial infarction could be found Multiple bleeding was observed in the pancreas

DISCUSSION

As there had been no doubt about the clinical diagnosis of major pulmonary embolism, the post mortem findings came as a complete surprise.

However on further consideration we have accepted the following explanation

Before the treatment started the patient had a thrombosis engaging the main veins of the entire right leg. As he improved markedly it must be assumed that most of the thrombus had been dissolved However enough material was left to constitute a considerable embolus which unfortunately was lethal even though it was under continuous dissolution. If the embolus had not totally disintegrated at the time of death, it is quite possible that lysis continued during the first hours afterwards. Consequently no solid material was found at autopsy The tragic fact that the patient succumbed in spite of successful thrombolysis was probably due to the persisting haemodynamic changes signs of which were to be seen at autopsy These changes together with hypoxia and—most certainly—a considerable stress were evidently too great a strain for this fairly old man.

The hypothesis of a continuous and finally total lysis of a lethal embolus is supported by the findings of Gajewski (1) In his report of a lethal case of pulmonary embolus during thrombolytic therapy he described the embolic material as gelatinous half liquid and thus partially lysed

As active fibrinolytic therapy is being used more and more in patients with thromboembolic disease (2 3 4 5) we find it important to point out this possible change in a formerly distinct pathology

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OVARIAN CYSTS IN WOMEN ON CHRONIC INTERMITTENT HAEMODIALYSIS

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Abstract In consecutive material of 42 female patients, who were treated with intermittent dialysi for chronic renal failure between May 1964 and Nov 1971 l developed ovarian cysts. The cysts were found only among the 29 women, who had menstrual periods from commencement of dialysi (16 cases) or who started menstruating on dialysi after period of secondary amenorrhoea (13 cases) N cysts were found i the remaining 13 patients, who had amenorrhoea throughout, and of whom 6 were all probability postmenopausal. Seven of the 21 patients with ovarian cysts had pronounced symptoms, necessitating acut surgery in 4 Fourteen asymptomatic cases were diagnosed at routine gynaecological examination, which was performed at regular intervals in all patients. There is strong evidence suggesting that the development of ovarian cysts was somehow related t the dialytic treatment and neither t the uraemic state nor to the nature of the primary renal disease. The mechanism by which dialytic treatment may be operational in the development of this hithert undescribed complication is discussed but no clear-cut explanation can be given.

In 1967 20-year-old woman, who had been on chronic intermittent haemodialysis for 39 months was acutely admitted because of severe abdominal pain. Emergency laparotomy was performed and a multicystic torquated left ovarian cyst containing haemorrhagic fluid was removed Histological examination showed a follicle cyst with haemorrhage The right ovary was slightly enlarged w th small cysts on the surface One month later the patient was readmitted with an identical clinical picture Acute surgery disclosed a rupt re of an 8 × 8 cm large cyst in the right ovary with haemorrhage into the peritoneal cavity and an oophorectomy was performed The histological diagnosis was a simple cyst"

This serious event led to a systematic prospective investigatio of the occurrence of ovarian cysts in women on chronic intermittent haemodialysis and a surprisingly high frequency of ovarian cysts was found It is the purpose of the present paper to discuss the nature of this complication which does not appear to have been reported before in the literature.

MATERIAL

Patients on chronic haemodialysis

The material consists of 42 consecutive female patients who were taken into chronic haemodialysis between May 1964 and Nov 1971

Renal disease The diagnosis of renal disease was established by conventional clinical and radiological criteria supplemented by histological examination of renal tissue obtained by biopsy and/or at bilateral nephrectomy With due reservation for the well known fact, that a histological diagnosis may be difficult in maximally contracted kidneys the diagnoses were as follows chronic glomerulonephritis 16 chronic interstitial nephropathy 16 *(largely comprising cases with analgesic abuse), polycys*tic kidney disease 4 and in the remaining 6, nephrosclerosis, thrombotic thrombocytopenic purpura, Alport disease, medullary sponge kidney congenital rological abnormality and bilateral traumatic renal lesion

Age at start of dialysis averaged 40 years (range 17 90).

Menstrual periods Si of the 4? women, verage age 46 years (range 45–50) had amenorrhoea before and during dialysis, and were in all probability postmenopausal Among the remaining 36 women, average age 38 years (range 17–49) 16 had menstrual periods before and during dialysis, 13 w th secondary amenorrhoea resumed normal menstrual period following an average of 3 months on dialysis (range 1 10), whereas 7 remained amenorrhoeic throughout Thus, 29 of the 36 premenopausal women had menstrual period on dialysis

Control material

The control material consisted of 27 randomly selected non-dialysed women ith severe renal failure Endogeno ou creatinine clearance averaged 11 ml/min (range ..–20).

Renal disease The diagnosis of renal disease wa established by clinical criteria supplemented by renal biopsy The diagnoses were chronic interstitial nephropathy (mostly due to analgesic abuse) 16 chronic glomerulonephritis 9 polycystic kidney disease 1 and *nephrosclerosis 1*

Age at gynaecological examination eraged 41 years (range 20–5)

Menstrual period Among the 27 women 15 (56%) had regular menstrual periods. Of the remaining 1 amenorrhoeic women 6 (average age 47 years) were in all probability postmenopausal

METHODS

Dialysis procedure The patients w re dialysed w th Kiil Gambro or Gambro-Lundia dialyzers for an erage period of 10 hour (range 9–1) twice week They re ceived a diet containing on an average 0.8 g protein/kg b wt. and sodium, potassium and fluid intake w s re stricted according t urinary losses N patient had sustained hypertension. Twenty had been bilaterally nephrectomized during dialysis.

The patient were heparinized during dialyses by single injections of heparin the return-line from the dialyzer under repeated control of clotting time which varied from 15 t 60 min. The total dose of heparin pe dialysis ranged from 15 000 to 25 000 IU Apart from occasional increases in the olume flow and duration of menstrual periods, other haemorrhagic complication were exceedingly rare.

G naec logic l examination of the patient on chronic haemodialysi w performed at start of treatment at regular ntervals during treatment (at least every 6 month) nd t presence of symptoms In the control material only one xamination w carried out

RESULTS

The control material of 27 severely uremic women ovarian y t were found

In the 4 haemodialysed patient no ovarian cyst were diagnosed t the first examination at the start of treatment

However n the course of chronic haemodialysis this diagnosis wa made n 1 of the 4 women Based on the way the diagnosis was made these 1 patients were divided into 3 groups (Table I).

Group I 4 cases Operation because of ruptured o arian cyst In all cases the histological diagnosis was follicle cysts or simple cyst " with haemor rhage

Group II 3 cases A ute abdominal pain and the finding of ovarian cysts at the gynaecological ex amination The complaint disappeared on conservative treatment.

Group III 14 cases These patients had no ymptoms and the diagnosis was made at the regular gynaecological examinations.

The following case histories are typical examples of the clinical course in the three groups.

CASE REPORTS

Case 1 group I

A 37-year-old woman with chronic glomerulonephritis, admitted in June 1969 in terminal renal failure The patient wa dialysed twice week for 14 months (143 dialyses). In Oct. 1970 she w s transplanted with necrokidney Two months later she was discharged with an endogenous creatinine clearance of 50 ml/min

G naecological data Menarche at the age of 11 She had alw ys been regularly men truated without deli eries, abortions or gynaecological complai ts. She had never been treated with oestrogens or progesterone At start of the dialysis treatment gynaecological xamination wa normal During dialysi the menstrual period continued but the indi idual periods were stronger and of longer duration. During the 38th haemodialysis treatment (4 month after start) the patient developed severe abdominal pai

At laparotomy a 5 × 5 cm large ruptured cyst in the right ovary and about 700 ml blood in the peritoneal cavity were found A partial resection of the ovary was performed Histological examination showed simple cyst One month later—in the middle of a menstrual period—an *identical clinical picture developed during the 45th* haemodialysis treatment. At gynaecological examination a 7 7 cm large cyst in the left ovary wa found and a left salpingo-oophorectomy w s performed The ovary contained non-ruptured cyst of the above mentioned size Histological examination showed follicle cyst with haemorrhage

Case 2 group II

A 26-year-old woman, admitted n Oct. 1968 in terminal uraemia due to chronic glomerulonephritis

In J ly 1969 after 9 months of haemodialysis, kidney transplantation wa *performed with kidney from the* patient brother (A-match) The postoperative course wa w thout complication and the patient is still doing well with an endogenous creatinine clearance of 60 ml/min normal BP and protein-free urine

G naecological data Menarche at the age of 13. Regularly menstruated until month before the above mentioned admi sion when the patient became amenorrhoei She had no deli ne or abortions, and no previous gynaecological complaint She had never been treated with sex hormones. Gynaecological examination at the start of haemodialysis treatment was normal. One month after the beginning of the haemodialysis treatment the patient became regularly me struated The day after the 16th haemodialysi treatment (2 month after start) the patient had pain the right iliac fossa She w in the middle of menstrual period nd gynaecological xamination showed an enlarged cystic right ovary The pai disappeared on conservati treatment During the following 6 months normal conditions were found at bi-monthly

Table I *Gynaecological data in 42 women on chronic intermittent haemodialysis*

	Groups with ovarian cysts				Without ovarian cysts
	I	II	III	Total	
No. of patients	4	3	14	21	21
Duration of dialysis period (mo.)	31 (14–59)	17 (7 38)	13 (1–31)	17 (1–59)	5 (1–27)
Age at start of dialysis (y.)	31 (17–45)	31 (24–46)	35 (18–49)	33 (17–49)	40 (28–50)
Duration of dialysis until signs of ovarian cysts (mo.)	20 (4–32)	15 (2–35)	7 (1–18)	10 (1 35)	
Regular menstrual periods throughout (no. of pats.)	3		7	1	4
Secondary amenorrhoea with resumption of periods during dialysis (no. of pats.)	1	1	7	9	4
Amenorrhoea throughout					13
Hormonal treatment during dialysis period			6	6	3

gynaecological examinations. One week before transplantation the right ovary was again cystically enlarged but the patient had no subjective complaints. Gynaecological examinations 3 and 18 months after transplantation were normal.

Case 3 group III

A 36-year-old woman admitted in May 1969 in terminal uraemia due to chronic glomerulonephritis.

After 12 months of chronic intermittent haemodialysis the patient received necrokidney. Due to thrombosis of the renal artery the allograft was removed 5 days after transplantation and the patient returned to chronic intermittent haemodialysis, the total period of dialysis being 29 months.

Gynaecological data Menarche at the age of 14. Always regularly menstruated. Two normal deliveries, 1959 and 1961. In 1966 an abortion was performed due to the chronic kidney disease. No sex hormone therapy was given. Gynaecological examination at the beginning of the haemodialysis treatment was normal. Routinely performed gynaecological examination 6 months after the start of dialysis (i.e. after 48 dialyses) showed an ovarian cyst on the left side. This cyst was found at later gynaecological examinations but was of varying size. After 28 months of treatment (corresponding to 224 dialyses) large cyst was found in the right ovary.

The patients with ovarian cysts were encountered in all types of renal disease. There was no preponderance in patients with polycystic kidneys.

All 1 patients with ovarian cysts were menstruating when the diagnosis was made. Among the 21 women without ovarian cysts only 8 were menstruating during the dialysis period.

The duration of the dialysis period was on an average 17 months in the group with ovarian cysts and 5 months in the group without cysts.

I all patients in groups I and II (Table I) the abdominal pain arose during the haemodialysis procedure or on the following day. Six of the 1 patients with ovarian cysts and 3 of the 8 menstruating patients without ovarian cysts were for short periods treated with oestrogens and/or progesterone largely for contraceptive purposes.

Twelve of the 1 women with ovarian cysts were transplanted all but one with necrokidneys. Four died shortly after the transplantation but in the remaining 8 patients no ovarian cysts were found at gynaecological examinations performed 7–.4 months after grafting.

DISCUSSION

At laparotomy Vara and Niemineva (3) demonstrated dysfunctional ovarian cysts with a frequency of about 7% in a large material. The incidence of symptomatic ovarian cysts is in all probability much lower although we ha e not been able to find exact figures pertinent to this question.

I the present material 21 of 42 consecutive female patients on chronic intermittent dialysis had ovarian cysts demonstrated either at regular gynaecological examination or by symptoms. The frequency of *asymptomatic and symptomatic cysts* was 1/42 (50%) in all women 1/36 (58%) in the premenopausal women and 1/29 (7?%) n the menstruating women. The frequency of *symptomatic* cysts was 7/4_ (17%) in the whole material 7/36 (?0%) in the premenopausal women and 7/29 (4%) in the menstruating women. Th frequency is so high that a further investigation of a possible rela-

tion to the primary renal disease to the uremic state and to the dialytic procedure seemed relevant

With respect to the primary renal disease it is pertinent that the presence of cysts was randomly distributed between patients with different kidney diseases Twenty of the 4? women were bilaterally nephrectomized but there was no preponderance of cystic disease of the ovaries in the nephrectomized group. As regards the possible role of the uraemic state per se the following findings seem to be relevant

1) No patients in a control material of 77 severely uraemic but non-dialysed women had ovarian cysts

) No cysts were found at routine gynaecological examination *at commencement* of dialytic therapy in the 42 dialysed patients

3) In 8 patients, in whom cysts were demonstrated during dialysis and who were successfully transplanted the cysts could not be demonstrated at follow-up examination after grafting

The above mentioned findings indicate that the development of cysts is related neither to the under lying renal disease nor to total nephrectomy nor to the uraemic intoxication per se. The evidence that the development of cysts is, somehow related to the dialysis treatment therefore appears convincing

Concerning the pathogenetic mechanism for the development of ovarian cysts in haemodialysed women two factors may be considered 1) The ect of heparinization.) A hormonal imbalance s regards heparinization, it is striking that ag vation of symptoms in the seven symptomatic cases did always occur either during the haemodialytic procedure or shortly afterwards In the six instances where surgical intervention was deemed necessary (two women being operated on twice) fresh *haemorrhage* was always found in the cysts There is thus little doubt that heparinization may *aggravate* symptoms by causing haemorrhage into preformed cysts but is highly unlikely that heparinization may be operational in the *development* of cysts Despite a careful perusal of the literature we have found no mention of the development of ovarian cyst in menstruating women who were anticoagulated for reasons other than haemodialysis.

As regards the possible role of a hormonal imbal ance somehow induced by haemodialysis, it is worth mentioning that all l cases of ovarian cysts developed among the ?9 patients who were menstruating during dialysis either from commencement of treatment (16 cases) or after a period of secondary amenorrhoea (13 cases) No ovarian cysts were observed in the remaining 13 women, of whom 6 were in all probability postmenopausal whereas 7 may represent cases of secondary amenorrhoea not relieved by dialysis

It must be of interest to know whether the menstruating women had ovulatory cycles or not. In our opinion there is considerable evidence in favour of anovulatory cycles 1) Measurements of basal temperature in a few of the patients of the present material as well as in a study of haemodialysed menstruating women since 1971 indicates that the cycles are anovulatory) Pregnancy is exceedingly rare in haemodialysed premenopausal women. 3) Measurements of plasma follicle-stimulating (FSH) and luteinizing hormones (LH) (see below) also favour the hypothesis of anovulatory cycles.

We found that the clearance of plasma oestradiol in conventional dialyzers is exceedingly low usually less than 1 ml/min of the non-protein-bound fraction. This finding taken together with the measurements of plasma FSH and LH indicates that depletion of oestradiol and/or progesterone from the plasma during haemodialysis resulting in an increased secretion of FSH and LH is hardly of significance

Ølgaard et al (_) found plasma FSH and plasma LH values within normal limits but without cyclic variation in premenopausal women dialysed in our department since 1971 The FSH/LH ratio was however high and not low as found by Abraham et al (1) in otherwise normal women with anovulatory cycles Whether this relatively high FSH/LH ratio can explain the development of cysts in our patients is uncertain.

The histological studies of the ovarian cysts removed in six ovaries in four of our patients showing either follicle cysts or "simple cysts" is of no aid in understanding the mechanism of the development of ovarian cysts

We are thus still in complete ignorance about the pathogenesis of ovarian cysts in our haemodialysed women The chief purpose of the present paper is therefore to present this hitherto undescribed complication to haemodialysis in order to see whether other treatment centres can retrospectively or prospectively verify its existence If so a much more careful investigation about the pathogenetic mechanism becomes highly relevant

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EPIDEMIOLOGY OF RENAL STONES IN A MIDDLE AGED MALE POPULATION

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Abstract The natural history of uppe urinary tract stones has been studied retrospectively i 49–50-year-old men in an urban population. The prevalence of stones in 2 322 men was found to be 13 7% with the highest incidence of onset of the disease during the fifth decade. Recurrences had occurred i 42% of all cases, the frequency increasing with the observation time. On some occasion 23% of the patients had been admitted to hospital and 12.3% had been operated on, 94 5% of all stones passed spontaneously A family history of kidney stones was significantly more common in stone patients than in healthy controls, and patients with family history of stones were more prone to early and repeated recurrences. It is suggested that the raised incidence of stone disease in some families may be attributed to environmental rather than genetic factors. This could be of importance for prophylaxis. Analysi of hospital admission rates supported previous findings of steady rise in stone incidence. The ad vantages of population studies for comparative analyses are pointed out.

The incidence of urinary tract stone disease has never been established precisely in any but the smallest communities (20) Comparative analyses of hospital admission rates indicate considerable temporal variations, with a steady rise in the incidence of upper urinary tract stones during this century (3) and marked geographic variations (3 6) Such differences may be attributed to ethnic socio-economic or nutritional factors (3) but there s a need for further information on dietary climatic and racial influences in the occurrence of urinary tract stone (7) The natural history of renal stones in patients admitted to hospital has been the subject of thorough investigations and these reveal among other things a high recurrence rate (24 29). Hospi tal admission rates can however be influenced by factors other than the incidence of the disease and cases examined in hospital may not fully represent uncomplicated stone disease It was therefore considered worthwhile to perform an epidemiologic study of stone disease in the population Such a study might also disclose groups with divergent clinical pictures, e g with a high recurrence rate where further investigations could provide additional information on the etiology of renal stones

This study reports a survey of five consecutive age classes of middle-aged men in whom the incidence and natural history of stones in the upper urinary tract have been investigated

MATERIAL AND METHODS

During 1970–73 health examination survey was offered all men living in the city of Uppsala, Sweden, who were born in 1920–24 (1). A total of 2 322 men were examined, a participation rate of 83 9% This investigation included detailed self-administered questionnaire according t Collen et al (8). One question concerned kidney stones and read. Have you ever suffered from renal colic?" All patients who answered affirmatively were recalled for detailed interview Only patients with history of stones in the upper urinary tract were included but no effort was made to distinguish between kidney and ureteric stones. The term kidney stones is, in thi paper used synonymously with upper urinary tract stones.

Only patients who at this nterview fulfilled at least one of the following four criteria were accepted for the present study 1) Spontaneous passage of stone noticed by the patient 2) Operative removal of kidney or ureteric stones. 3) Roentgenologically demonstrated stones of the upper urinary tract. 4) Characteristic clinical findings judged by physician at the time of symptoms. The patient s statements about hi ymptoms were accepted in those cases included according to criterion 4 even when records from the examining physician were not available

The clinical history was broken into separate units called "incidents (29). Each incident represented stone or group of stones with continuous ymptoms. If symptoms ere not terminated by observed passage of stone, all symptoms during the following year were attributed to the same incident Also succession of stones passed at short intervals without roentgenological evidence of stone-free urinary tract in between was classified as only one incident. After symptom-free period of one year

Table I *Total number of stones in 318 middle-aged men in relation to the age at onset of symptoms*

Age at onset (y)	Total no of stones							Total no. of pats.	
	1		3	4	5	6–9	10	*n*	%
19		1						3	0 9
20–24	5	1		3			3	16	5 0
25–29	6	6	6		1	3	3	25	7 9
30–34	27	4	3			2	4	44	13.8
35–39	39	18	5	4		1	6	75	23 6
40–44	32	13	10	3	1		5	64	20.1
45–49	74	6	6	4		1		91	28 6
Total no of pats.	185	49	3	16	6	9	1	318	100.0

new symptoms were considered to be due to the formation of a new stone even if indisputable evidence of stone passage not had been produced In the text the term "stone" generally denotes the concept of "incident"

All data concerning operations were collected from the original hospital record Cystoscopies for purely diagnostic purposes were not included, nor were unsuccessful attempts at endoscopic stone extraction Serum calcium and uric acid estimations and qualitative test for cystine in urine (Brand test) were performed in almost all cases. Detailed investigations for diagnosis of stone etiology were carried out only when relevant to the clinical picture e.g. in patients with many recurrences.

Information regarding the occurrence of kidney stones among first-degree relatives was obtained through postal questionnaire to all stone patients The same questionnaire was also sent to control group consisting of all other men in the city born in 1924 reported to be stone-free at the health survey 309 stone patients (98.2 %) and 393 controls (91 5 %) replied to the inquiry This information was accepted without further attempts at confirmation A family history of kidney stones was considered to exist if one first-degree relative had had a kidney stone even if the informant did not have full knowledge of all his relatives. On the other hand absence of family history of stones was accepted only if the informant could negate the occurrence of stones in all his first-degree relatives Consequently if he did not possess information concerning all relatives, he was not included in the calculations regarding family occurrence of stones Adequate information was given by 269 stone patients (85 %) and 320 control (74 5 %)

With the aid of postal questionnaires, a small study was also made of the occurrence of kidney stones among the non-participants in the health survey Of 66 individuals born in 1924 who had not taken part in the examination, 49 answered this enquiry This information was also accepted without further investigation

Patient data concerning in-patient care in the city of Uppsala during 1969 was collected with the National Social Welfare Board s permission, through discharge information on each individual from the University Institute of Social Medicine.

Student's *t* test the χ^2-test with Yates correction for small populations, and Fischer exact test for 2 tables were used in the statistical analyses. Accepted level of significance was $p<0.05$

RESULTS

Stone prevalence

Of the 2322 men aged 49–50 who participated in the health survey 343 (14 8 %) answered the question. Have you ever suffered from renal colic?' affirmatively This could not be substantiated at the following interview in 25 cases of which 11 were not available for investigation In 9 cases the diagnosis was questionable and 5 individuals had mistakenly acknowledged that they had had renal colic The remaining 318 men represent 13 7 % of the investigated male population in these age groups In 236 cases the diagnosis was considered verified by observed passage of stones operation or X-ray (criteria 1–3) on at least one occasion In the remaining 82 individuals the diagnosis was regarded as highly probable even though no stone had been visualized (criterion 4) Of these patients 73 had a single stone and thus in only 9 patients with recurrences were stones never visualized

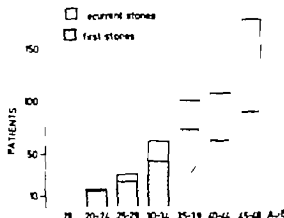


Fig 1 Total number of patients with kidney stones during each 5-year period No patient is included more than once during each period

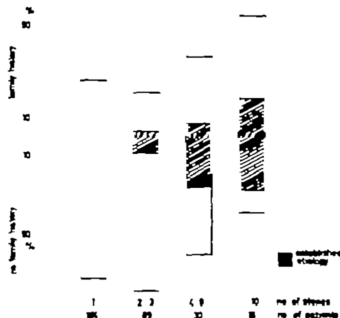


Fig. 2. Family history of kidney stones in relation to number of stones. Frequency of recognized etiology of stone formation in relation to family history and number of stones. Only patients with full information regarding stone disease in relatives are included.

Among the non-participants in the health survey who answered a postal enquiry 20.4% reported kidney stones. This figure is not significantly different from that of the participants.

Table I shows that kidney stones were rare before the age of 20 but after 30 the number of stone patients rapidly increased. This increase continued up to the age of 50. The number of patients with recurrences also increased with time. The highest stone incidence consequently occurred in the 5-year period preceding the health survey, when 179 individuals had at least one stone, which corresponds to 7.7% of all men in these age groups (Fig. 1).

Possible etiology of stone formation

Although it was beyond the scope of this study to establish an etiology of stone formation in every instance, a possible cause was found in 30 cases (9.1%), mainly patients with several recurrences. Among the diagnoses were nine operatively verified cases of hyperparathyroidism, three cystinurias and three medullary sponge kidneys.

Family history of stones

Kidney stones in at least one first-degree relative (family history of stones) were present in 29.4% of stone patients and 15.3% of stone-free control individuals from the same health survey. This difference is highly significant ($p<0.001$). A family history of stones was particularly common in patients with multiple stones (≥4). Fig. 2 shows that this raised incidence of family history in multiple stone formers came entirely from patients with no known cause of stone formation, whereas if a possible diagnosis was established, a family history of stones was not more common in patients with recurrences.

Recurrences

Altogether 133 patients (42%) had experienced at least one recurrence of stone by 50 years of age. The risk of relapse increased continuously, from 31.5% after 5 years to 72% in patients who had had their first stone attack more than 20 years previously.

Patients with a family history of kidney stones had on an average experienced more stones by the age of 50 than those without stones among relatives. This could not be explained by any difference in age at the onset of stone disease, nor was the total number of patients with recurrences significantly different (Table II). However, multiple recurrences were significantly more common in patients with a family history of stones and the early recurrence rate in these patients was significantly increased. Whether previous stones had been operatively removed or passed spontaneously did not seem to influence the recurrence rate.

Medical care

This material derives from a city population with good access to emergency medical care and with

Table II *Comparison between patients with and without family history of kidney stones with regard to age at onset of symptoms total number of patients with recurrences and frequency of multiple and early recurrences*

Only patient with full information regarding stone disease in relatives are included

	Age at onset (y)	Total recurrence rate (%)	≥3 recurrence (%)	Recurrence (%) In 5 y	Recurrence (%) In 10 y
Family history ($n=79$)	37 9	46 9	27 9	48.2	56.8
No family history ($n=190$)	38 3	41..	12.6	25.8	32 4
Significance of difference	ns	ns	$p<0\ 01$	$p<0\ 001$	$p<0\ 001$

ns = not significant.

local traditions favouring early hospital attendance in case of acute symptoms The medical care for these patients is presented in Table III In cases without apparent passage of stone a physician s diagnosis was required if the patient was to be included in the present study This is however a minor reason for the high rate of medical care since very few persons were omitted for not fulfilling this criterion.

The proportion of patients treated at hospital on some occasion increased with the number of stones and only a small number of patients with repeated recurrences were treated exclusively in private practice It was at the same time evident that patients with experience of stones from several recurrences attended medical care less often particularly in cases of early spontaneous passage of the one

Ope ation

In all 811 stones were reported 47 stones (5 5%) in 39 patients (12 3%) were removed at operation Spontaneous stone passage thus terminated 94 5% of the stone incidents The number of operations is too small to permit any detailed separation according to surgical technique Half of the operations consisted of ureteral surgery almost exclusively ureterolithotomies. Operations on the kidneys and endoscopic stone extractions were equally common and made up the other half

There was a tendency for the operation rate to increase with age Before the age of 40 3.8% of stones were removed at operation compared to 6 9% after 40 years of age Another finding was a lower incidence of operations in patients with multiple stones (≥4) In these patients only .8% of all stones required operation whereas 9.. % of patients with fewer stones were operated on

Hospital statistics

In order to obtain an estimate of the development of the incidence of kidney stones in this region statistics for the two hospitals serving the city of Uppsala were analysed for 1969 During this year a total number of 138 patients of both sexes were admitted to hospital because of stones in the upper urinary tract. This represents 95 individuals per 100000 population. The age distribution and the proportion of operations for the men in this hospital material are compared in Fig 3 with the information given by the participants in the health survey This comparison shows a substantial increase in the hospital admission rate in the younger age groups Among 25–29-year-old men hospital admissions during 1969 were five times more common than among the participants in the health survey at the corresponding age The figure also shows continually increasing rates with age for hospital admissions and operations for the men in the health survey During 1969 however hospital admissions for upper urinary tract stones were equally common in the age groups 25–29 and 45–49 for males in Uppsala.

Table III *Medical care for kidney stones in male stone patients in an urban population*

	%
Admitted to hospital at least once	.3 0
Hospital out-patients never admitted	55 0
Physicians outside hospital only	17 9
No medical care	4 1
	100 0

DISCUSSION

As far as accuracy in diagnosis is concerned there are obvious advantages associated with only investigating patients admitted to hospital However for epidemiological purposes less rigid criteria for diagnosis sometimes have to be accepted A considerable proportion of ureteric stones verified by X-ray pass spontaneously shortly after the onset of symptoms without apparent stone passage (24) It was therefore considered justified to include symptomatic cases without definitely visualized stones in this investigation These cases constitute about 25% of the total mainly patients with single stones. This proportion between objectively verified and mainly symptomatic cases agrees fairly well with other reports concerning clinically typical cases of stones in the upper urinary tract (9 16 24)

In judging the natural history primarily from information given by the patients there are possibilities of over as well as underestimating recurrences *Since the majority of stones in the ureter pass within weeks of the onset of symptoms (10 24)* and in this investigation repeated symptoms within one year were attributed to the same stone, the risk of introducing false positive recurrences seems small but some early recurrences (within the first year) may not have been recognized A recent investigation has shown that the number of completely asymptomatic stones is probably small and that the value of repeated X-ray control for their discovery is limited (22) Most recurrences are therefore believed to have been properly noticed by the patients.

Comparisons between compilations of hospital admissions for urinary tract calculi in diff rent parts of Sweden (11) and other parts of Europe and the

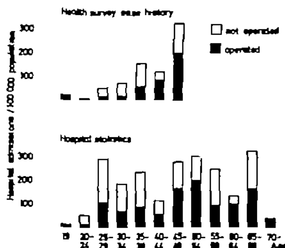


Fig 3 Hospital admissions for kidney and ureteric calculi in men in Uppsala. Case history information given by the participants in the health survey and hospital statistics from 1969 Numbers are expressed per 100 000 population in the respective age groups.

USA show large variations with a rather low hospital admission rate in the province of Uppsala (21) Compared to other population studies the present prevalence of kidney stones in middle-aged men (13 7%) does not support the presumption that the low hospital admission rate reflects a low stone incidence (Table IV). In fact, only 23% of these stone patients have on any occasion been admitted to hospital in spite of excellent facilities for such admissions. This clearly demonstrates the difficulty in using hospital admission rates as a measure of the incidence of this disease The results from the three health surveys of middle-aged men in the different parts of Sweden presented in the Table indicate

Table IV *Prevalenc of uppe urinary tract stones in men in population studies (diagnosis based on clinical picture)*

Country	Group	Prevalence (%)	Reference
Denmark	Doctors, all ages	12	(16)
USA	All ages (estimated figure)	4.2	(23)
Great Britain	Royal N vy 50-year-olds (estimated figure)	3.5	(4)
Norway (Oslo)	50–59-year-olds	11 6	(27)
Sweden			
Gothenburg	Health survey 50-year-olds (only visualized stones)	6.8	(25)
Malmo	Health survey 55-year-olds	4 1	(13, 14)
Uppsala	Health survey 49–50-year-olds	13 7	Present study

however that local circumstances may considerably influence the incidence of kidney stones.

The rapid increase reported previously (3) for the incidence of stones in the upper urinary tract seems to be continuing Since earlier population studies are lacking, these comparisons have to be based on hospital admission rates. Hedenberg (11) in an analysis of hospital statistics from the province of Uppsala, found an annual incidence of stones in the upper urinary tract of 19 per 100 000 inhabitants during 1943–46 The present study of the same hospitals 25 years later shows a five-fold increase. The principles for admission do not appear to have changed at these hospitals in recent years and this increase therefore may reflect a true rise in stone incidence The rise in incidence over time and the possibilities of large geographic variations have to be considered in analyses of stone occurrence e g. in the selection of all sorts of control materials.

In analyses of cross-sections of the population it is often found that the commonest age at onset of stone disease is during the third or fourth decade and a decline is common in middle age (1 2 4 5 17 18 29) However it is at the same time apparent that for the men in this study the incidence increased continually over the years and any decline would have to occur after the age of 50 This rising incidence during life may be related to aging as such but also to prolonged exposure to environmental factors If the latter are predominant and of increasing importance younger age groups will be affected to a greater extent This may explain why the incidence is about the same in the different adult age groups in the population as a whole when the risk for the individual is rising so markedly

In this study more than one eighth of all men had been affected by kidney stones by the age of 50 The number of recurrences also increased with time and of patients with a disease of more than 20 years duration 77% formed another stone Thus the formation of kidney stones is not a singular event but a process in which the risk of recurrence remains for many years Given enough time most patients seem to relapse ... large series have also failed to distinguish ... apt to have recurr... stones from single s... rs as far as bloch... of serum and ... ition of ... in- fection ... y are ... Apart from ... whom the etio ... e ... ion ... ed and ... a ... for k

tients with an increased risk of early and frequent recurrences partly because in these cases prophylactic measures may be indicated and partly in order to gain a better understanding of idiopathic stone disease In this study the patients with a family history of stone disease from an epidemiological point of view constituted such a group with a greatly increased risk.

The observed co-variation between a family history of stones and the clinical picture with repeated recurrences may indicate that patients with stones among their relatives have features in common which make them liable to stone formation. Only a few rare causes of stone formation are definitely known to be inherited and probably constitute too small a number to significantly affect the calculations in this study That a family history of kidney stones is more common in stone patients than in control individuals has been shown before (17 19 23) and is confirmed in the present study It has previously been considered unlikely that the data in these respects are significantly biased by patients with stone disease having a heightened interest in stones amongst their relatives (19 23). Whether a familial occurrence is due mainly to genetic or environmental influence is not known. Resnick et al (23) found evidence compatible with the hypothesis that the tendency to form calcium oxalate stones is regulated by a polygenic system. However Transbol and Frydendal (26) suggested that environmental factors might be involved and be responsible for their finding of a higher percentage of affected male relatives among patients with idiopathic hypercalciuria than when no metabolic cause for stone formation was discovered The importance of non-genetic household factors was also stressed by White et al (28) who found an increased urinary calcium excretion in spouses of stone-formers

Though some inherited predisposition may also be involved it seems that environmental factors at least in some instances can contribute to the formation of stones Epidemiological studies might be useful for further work on the identification of such factors in the environment the ultimate goal being to provide a rational prophylaxis and treatment of idiopathic stone formation

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ADULT TYPE OF POLYCYSTIC KIDNEY DISEASE IN A NEW-BORN CHILD

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Abstract. A case of polycystic kidney disease in new-born child is reported Renal cortical necrosis due to asphyxia was the cause of death The histopathological picture and heavy family history support the rare diagnosis of polycystic kidney disease of adult type in new-born child.

Polycystic disease of the new-born or infant is represented by a variety of gross and microscopic pictures Most children with polycystic disease are still-born or die soon after parturition On rare occasions polycystic kidney disease has been diagnosed in early childhood in families with the dominantly inherited adult form of polycystic kidneys (? 5 6).

The following s a presentatio of a new-born child with polycystic kidneys. The adult type of polycystic kidneys had been manifest in four earlier generations of the family

CASE HISTORY

A 22-year-old woman had right-sided cystadenoma simplex resected during the 4th month of her first pregnancy Two weeks before full term in Dec. 1972, she was admitted to hospital because of slight proteinuria Her BP was normal Twelve days later she developed toxemia, the fetal heart rate decreased, and cesarean section was carried out on the indication of impending asphyxia On exploration of the mother abdomen polycystic kidneys ere found The liver was normal on palpation The post operative course was uneventful

The new-born was girl mature and with no external malformations. She remained asphyctic and was taken to ward for intensive care. She had edema, passed no urine, and was found to be uremic on the following day Peritoneal dialysis was started but the child died two days after birth At autopsy polycystic kidneys and cortical necrosis were found (Figs. 1–4)

Family history

The mother had 5 years earlier had an ttack of left-sided flank pain and microscopic hematuria. Urography showed a small contrast defect in the left kidney and the episod was judged to be due to a passage of concrement The kidneys were normal-sized and not even in retrospect were there any signs of cysts A year after parturition she has undergone new urography which shows enlarged kidneys, length 18 cm, and the appearance of cysts of varying size. Her glomerular filtration rate is normal, but her concentration capacity i moderately reduced.

The mother has sisters, 27 and 19 years of age They are healthy so far but have not been examined The grandmother had polycystic kidneys diagnosed at the age of 40 developed uremia at 47 when she got renal transplant which is still functioning after 5 years. The grandmother s brother had polycystic kidneys and died from uremia t the age of 45

Another 6 members of th family are known to have had polycystic kidneys. the great grandmother 2 great aunts, great-uncle the grandmother grandfather and cousin. They all died from uremia between 40 and 50 years of age. Thus, at least 5 generations have been affected by this heritable disease The family pedigree s shown in Fig. 5

Pathology

The necropsy showed a left kidney slightly enlarged and right kidney of normal size total weight 30 g after fixation in 10% formali Afte stripping off the fibrous capsule, multiple cysts, the size of pinhead and containing transparent fluid were exposed on the cortical surface (Fig 1) In sections the cortex was mottled brown and yellow and engorged with blood the medulla was pale with well formed papillae (Fig. ?) There were no malformations of the pelves, ureters, bladder urethra or the genital organs There were no cysts to be found the liver which was of normal size, structure and consistency No congenital malformations were demonstrated in other internal or gans. Extensiv atelectases of the lungs were found bilat erally furthermore there were signs of general circulatory disturbance with generalized edema and acute blood stasis

The microscopical examination of the kidneys demonstrated bilateral cortical necrosis of irregular extension and with prominent interstitial haemorrhage from blood-filled capillaries. There were small spherical cysts, maximally 1– mm in diameter but generally much smaller almost e clusively localized in the cortex and considera-



Fig 1 The kidneys with capsules removed. Small cysts hardly visible, are present in the cortical surface Note the normal extent of fetal lobulation.

bly more numerous than could be macroscopically imagined. The distribution was characterized by groups of 5–10 cysts within the cortex the extension corresponding to groups of neighbouring nephrons, and interspaced by normal parenchyma (Fig 3). Many of the smaller cysts contained a peripherally located glomerulus (Fig. 4). There were no signs of dysplasia (dysontogenetic tissue) in the kidney sections studied

COMMENTS

At the turn of the century Köster (8) showed that the age distribution of polycystic kidney disease has two peaks one at the time of birth and one between 40 and 50 years of age (Fig. 6) The bimodality in age at diagnosis has been apparent whenever extensive series of cases have been collected and it has led to the view that there are two distinct entities

The polycystic kidneys in adults have been well defined from clinical pathological and genetic viewpoints (3 5). The heredity is autosomal dominant.

Polycystic kidney disease in new-borns and infants is not a homogenous pathological entity and there has been some confusion about the terminology and classification (2 4 10). Clinical pathologi-



Fig 2 Longitudinal sections through the kidneys Cortical cysts are hardly visible at this magnification



Fig 3 Characteristic finding of small collection of cyst in the cortex. The surrounding parenchyma is necrotic (htx–van Gieson 50)

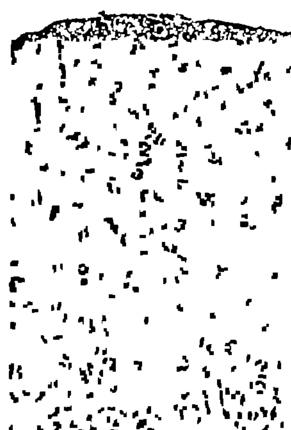


Fig 4 Corpuscular type of cysts with glomerular structures sible in the lumen. Note also the necrotic tubular epithelium especially in proximal tubules (htx van Gieson 140).

cal and genetic features have not always been correlated Thus Osathanondh and Potter classified exclusively on the basis of microdissection studies In a critical survey Bernstein (1) emphasizes that renal cysts in syndromes of multiple malformations and hypoplastic kidneys with cyst formation should not be confounded with infantile polycystic kidney disease The most typical pathological picture shows radially arranged fusiform or cylindrical cyst and these changes are usually combined w th cysts of the liver (7). This variety seems to be transmitted as a recessive trait manifested only in homozygotes Siblings are often affected but never the parent

Most studies on polycysti d sease seem to confirm the concept that the entities in childhood d ffer from that in adulthood Thus Lundin and Ol ne (9) in their study of 23 ew-born nfant nd c ldren found none w th fa- ly history in earlie genera tions. There are ery few report of polycystic kidney disease in early childhood in families with adult type of polycystic kidney One of these reports suggests the coincidence of an infantile type (associated w th hepatic fibrosis) and the adult type in the same family (6) Ferguson (5) was the first to describe a child 5 year-old with polycystic kidneys in family with dominant nheritance of the disease Blyth and Ockenden (2) described an interesting family with polycystic kidneys in a f ther and a 2-year-old girl The mother had a later preg nancy terminated in the 14th week and twin fetuses were found Microscopic examination re ealed few cort cal and medullary cystic (dules in both k dneys of both f tuses

There re many theorie of the path gene i of polycystic kidney b t the c uses of both adult and cf ntile f orm are till unknown The ysts in polycy tic k dney dise se n arise fr om any point along the nept r on (7) When they aris fr on cystic

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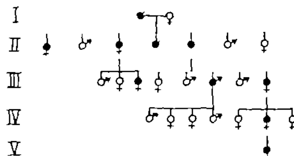


Fig 5 Family Pedigree ●=polycystic kidneys. Six of the individuals in the 4th generation (aged 19–30) have not been submitted to renal examination.



Fig 6 Polycystic kidney disease Age distribution in 239 cases (Köster)

dilatation of Bowman s capsule glomerular tufts are recognizable in the lumen Typical for the adult type is the spherical configuration of the cysts and in the earlier stages groups of cysts are surrounded by parenchyma of normal structure

The new-born child in the present report demonstrated a histopathological picture which might well be compatible with an early stage of adult type of polycystic kidneys With the heavy family history there can be no doubt about the classification of the kidney disease

The child died from anuria, which could hardly be due to the cystic disease. The cortical necrosis initiated by an intra and extrauterine asphyxia, seems to be the probable cause Very little is known about the early development of polycystic kidneys of adult type since clinical signs and symptoms are late manifestation Only autopsies of fetuses new-borns and infants from affected families can vide nformation when death occurs in intercur diseases

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THE EFFECT OF SPIRONOLACTONE (ALDACTONE®) AND METHYLDOPA IN LOW AND NORMAL RENIN HYPERTENSION

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Ab tract The effect on BP of 100 and 200 mg spironolactone/day has been compared with that of methyldopa, 750 mg/day and with combined treatment with both drugs in 32 patients with essential hypertension. The 28 patients who completed the entire investigation ere treated for 30 weeks, divided into 4 treatment periods and 4 placebo periods of equal duration. BP did not fall significantly during the nitial placebo period and at th end of each of the intervening placebo periods t rose to pretreatment levels A significant decrease in mean BP was found during the 4 treatment periods. A fall exceeding 14% was registered in 32% of the patients after methyldopa 750 mg/day In 50% of the patient after spironolactone 200 mg/day in 89% after combined treatment with both drugs, and in 29% after spironolactone, 100 mg/day Low renin hypertension was found in 9 of the 28 patients. The verage decrease in mean BP after spironolactone 200 mg/day methyldopa 750 mg/day and after combined treatment did not differ significantly between the low and the normal renin group. The rationale for using diuretics such as spironolactone or thiazide as the basic therapy I essential hypertension is discussed It is concluded that both are useful in the treatment of essential hypertension and might be used alone or in combination.

Diuretics of the thiazide group are commonly accepted and widely used as a basic therapy in the long-term treatment of hypertension, although these drugs may have profound metabol c side effects which have to be considered when they are used for several decades in subjectively healthy people with clinically diagnosed hypertension Recent investigations have shown that hypertensive patients may be grouped after their plasma renin activity (PRA) nd that whereas spironolactone does control the BP in low PRA hypertension, this is not so certain in normal and high renin hypertension (4 6 1.. 14 18 19)

The two main purposes of the present investigation have been: 1) To study the effect of spironolactone and methyldopa, alone and in combination and compared with placebo in the treatment of benign essential hypertension. 2) To study the effect of treatment in relation to levels of PRA

MATERIAL AND METHODS

Fifteen women and 17 men, aged 33–73 years, with the clinical diagnosis of benign essential hypertension were included in this study All of them had normal renal function and fundus hypertonicu grade I or II BP was measured each time ambulant under as identical conditions as possible with the same adjusted Hg-sphygmomanometer first after 10 min bed rest, then after 2 min of standing and finally after 10 knee-bendings, and the averages of these measurement being sed. The mean BP is defined as diastolic BP+1/3 of the pulse pressure

The entire investigation lasted for 30 weeks each treatment period of 4 weeks being followed in principle by 4 weeks of placebo (Fig 1) There were 4 treatment periods altogether and -week placebo regimen concluded the study The majority of the patients had not received antihypertensive treatment previously and in the remaining cases the hypotensive treatment was cancelled 3–4 weeks prior t the study

Three months after this experiment had been completed, after cancelling hypotensive therapy for the last 2 weeks blood samples were taken for the determination of PRA (21). The patients had been on normal diets and the samples were taken ambulant the first sample with th patient in pright position having walked around for two hours, and the second sample hours after 80 mg furosemide orally The upper normal level with the present PRA method is 1 3 ng ang I/ml/h, based on ambulatory measurement in more than 600 patients with benign essential hypertension without previous treatment (to be published). Patients with PRA level of 0 2 ng ang I/ml/h or less before and after stimulation with furosemide were classified as low renin hypertension patients Plasma aldosterone concentration was determined (22) the low ren group only to exclude primary aldosteronism

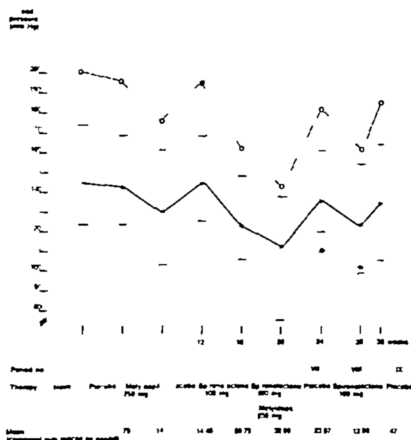


Fig 1 Mean BP in 28 patients with essential hypertension during the different treatment periods

RESULTS

[F]ig 1 shows the average BP for the 28 patients who [com]pleted the investigation before and after the [trea]tment periods The statistical evaluation of the [c]hanges in mean BP is given in Table I

The patient group as a whole showed a significant fall in mean BP in all the active treatment periods. The strongest effect was obtained when spironolactone 200 mg/day and methyldopa 750 mg/day were combined (S_2MD) It should however be observed that this period of combined treatment was not preceded by a placebo period so that the BP did not start from baseline levels The second best effect was obtained after spironolactone 200 mg/day (S_2) while there was no significant difference in the BP response between methyldopa 750 mg/day (MD) and spironolactone 100 mg/day (S) (Table I)

The interindividual variation in the BP response was considerable in all treatment periods but the effect was more uniform with S_2 than with MD A fall in mean BP exceeding 14% was registered in

Table Ia *Means and variations after four different treatments Absolute (mmHg) and percentage reduction (within parentheses) of mean BP from preceding placebo period*

MD = methyldopa (250 mg 3) S_2 = spironolactone (100 mg × 2) S_1 = spironolactone (100 mg × 1) S_2MD = S_2 + MD

	MD	S_2	S_2MD	S	Mean
$\bar{x}$	14 14 (9 75)	20 75 (14 41)	32 00 (22.36)	12.57 (9.38)	19.87 (13 98)
S.D	1[illegible]28 (8.27)	8.69 (5 61)	10 04 (6.75)	9 14 (6.93)	10 14 (6.95)

Table Ib *Analyses of variance of mean BP reductions induced by four different forms of treatment*

	D F	Sum of squares	Mean squares	F	Significance
Treatment	3	6 551 46	2 183.82	24 70	$p<0.001$
Patients	27	3 933.25	145.68	1.65	$p<0.05$
Errors of trial	81	7 160.29	88 40		
Total	111	17 645 00	158.96		

Significance tests on 6 individual comparisons Tolerance factor t ... confidence=90% conf.int. $=t.S.\sqrt{2/28}=5.53$ MD–S_1=−6.61 MD–S_1MD=−17.86, MD–S =+1.57 S_1–S_1MD=−11.25 S_1–S_1=+8.18, S_1MD–S_1=+19 43 All the comparisons except MD–S are significant, i.e. the differences are greater than the related confidence interval 5.53

32% of the patients after MD in 50% after S_1 in 89% after S_1MD and in 29% after S

In the low PRA group after S_1 the mean BP was reduced by an average of 22 6 mmHg (Table II) compared with 19 9 mmHg in the normal PRA group (Table III) the corresponding reductions after S_1MD were 34.3 and 30.8 mmHg These differences between PRA groups in the reduction of mean BP were not statistically significant. Neither were any such differences found after MD

The serum concentrations of kalium, urea, cholesterol and uric acid were determined in most patients before and after two months on S_1, the last month combined with MD (Table IV). In 26 patients the serum kalium concentration increased significantly ($p<0.01$) from mean 4 0 to 4.5 mEq/l. In two patients more than 5 0 mEq/l were found at the end of the treatment period with subsequent normalization after withdrawal of spironolactone In 17 patients the mean urea concentration increased significantly ($p<0.05$) from mean 38 to 49 mg/100 ml in three of these cases levels of 64–79 mg/100 ml were found at the end of the treatment period The slight decrease in cholesterol and increase in uric acid were not significantly different from the pretreatment levels

Side-effects

The most common side-effects were fatigue and dizziness in four patients while on MD in three on S_1 and in six on S_1MD After treatment with MD three patients developed nausea as did three after S_1 and three after S_1MD In four of the patients the treatment was withdrawn on account of these side effects, in one after S_1, in the other three after S_1MD

Table II *Change in mean BP (mmHg) after MD S and S MD in 9 low renin hypertension patients*

B before A=after furosemide (80 mg). Other abbreviations as in Table I

Pat. no.	Age (y)	Sex	PRA (ng ang.I/ml/h) B	A	BP during placebo period	Change in mean BP after MD	S_1	S_1MD
1	45	♀	<0 1	0.2	170/115	−14	−27	−34
2	61	♀	0 2	0 2	165/105	−24	−18	−47
3	53	♂	<0.1	0.2	165/105	−13	−17	−39
4	71	♂	<0 1	<0 1	213/120	−13	−29	−38
5	63	♂	<0 1	<0.1	198/117	− 9	−26	−35
6	70	♂	<0 1	0.2	220/120	−47	−22	−38
7	52	♀	0 1	<0.1	205/113	+ 7	−25	−24
8	57	♀	<0.1	<0.1	40/130	− 7	−26	−28
9	57	♂	<0 1	<0 1	198/98	− 9	−13	−26
Average	58.8		Low PRA		197/114	−14.3	−22 6	−34 3
S E.M						± 4 90	± 1 80	± 2 43
Significance						$p<0.05$	$p<0.01$	$p<0.01$

Plasma aldosterone levels were all normal, ranging from 16 t 62 pg/ml

Table III *Change in mean BP (mmHg) after MD S_1 and S_1MD in 19 normal renin hypertension patients*
Abbreviations as in Table II

Pat. no	Age (y)	Se	PRA (ng ang.I/ml/h) B	A	BP during placebo period	Change in mean BP after MD	S_1	S_1MD
1	63	♀	0.3	1.5	220/120	−34	−29	−39
2	44	♂	1.3	1.3	180/130	−10	−19	−19
3	58	♂	0 4	1.2	210/118	−27	−26	−35
4	57	♀	0 4	0.8	180/105	−18	−23	−27
5	50	♂	0.3	0.2	172/117	−23	−26	−12
6	51	♂	0.3	0 2	178/128	+ 6	−18	−45
7	67	♂	0.5	0 6	190/110	− 5	−20	−22
8	61	♂	0 9	0 7	207/118	−28	−10	−19
9	72	♀	0.3	0.3	212/110	−23	− 1	−36
10	48	♀	0.8	1 4	200/110	+ 1	−10	−34
11	66	♂	0.2	1.8	195/127	−16	−43	−39
12	63	♂	0.3	0.2	163/102	0	−12	−24
13	64	♂	0.8	0.6	183/112	−15	−24	−31
14	56	♀	0.2	0.5	215/135	−24	−12	−41
15	33	♂	0.3	0.2	163/103	0	−11	−19
16	52	♂	0 4	0.5	232/132	−24	−20	−30
17	53	♀	0 4	1 4	237/120	−14	−28	−33
18	60	♀	0.4	1.0	?08/115	−12	−33	−58
19	50	♂	<0 1	0.3	167/107	− 2	−13	−23
Average	56.2		Normal PRA		195/117	−14 1	−19 9	−30 8
S E.M						± 2 65	± 2.27	± 2.54
Significance						$p<0.01$	$p<0.01$	$p<0.01$

DISCUSSION

In most of the 28 patients who completed the trial the hypotensive response observed with S_1 was more pronounced than with MD while there was no significant difference between MD and S The BP l was greatest, however when S_1 and MD were together (S_1MD) Average falls in mean BP of ¹4% after S_1 and of 22 4% after S_1MD indicate that these regimens are alternatives in the medical treatment of essential hypertension.

Recent investigations have shown that patients with essential hypertension form a heterogeneous group in terms of PRA levels low PRA being found in about 15–30% of all patients with this clinical diagnosis (5) Although increased production of aldosterone or other mineralocorticoids such as corticosterone deoxycorticosterone or 18-hydroxy deoxycorticosterone has been found in some patients in the low PRA group the great majority have normal levels of these steroids (5 11) In some

Table IV *Serum concentrations of kalium urea cholesterol and uric acid before and after S_1 for 2 months combined with MD during the la t month*
Abbreviation as in Table II

Kalium (mEq/l) B	A	Urea (mg/100 ml) B	A	Cholesterol (mg/100 ml) B	A	Uric acid (mg/100 ml) B	A
4.0 (3 1–4 6)	4 5 (3.8–5 4)	38 (21–56)	49 (30–79)	300 (227–400)	282 (175–386)	6.2 (4.0–11.6)	7 4 (4.8–11 4)
=26	26	17	17	14	14	14	14
Significance	$p<0.01$		$p<0.05$		N.S		N.S

cases however there may be a relative or inappropriate mineralocorticism in relation to the sodium and water balance (9 15) or an increased sensitivity to normal steroid levels

Several investigations indicate that patients with low PRA hypertension respond favourably to treatment with spironolactone (6 8) as in the case of primary aldosteronism (18)

Thus in a double-blind investigation, Carey et al (6) found a strong effect of spironolactone in all cases but one in the low PRA group whereas no significant effect on BP was found in the normal and high PRA group while other authors have found that spironolactone may reduce high BP in normal PRA hypertension as well (12, 14) Adlin et al (2) found that the hypotensive response to spironolactone was the same as that to hydrochlorothiazide in the low PRA group, indicating that these therapeutical regimens might be equivalent

In the present investigation the average decrease in mean BP after S_4 in the low PRA group with normal plasma aldosterone levels was equal to that in the normal PRA group Neither did the hypotensive response to S_4MD differ significantly between the two groups

The varying hypotensive response observed with spironolactone in different investigations when related to PRA levels, might be due to several factors such as differences in the doses used duration of the treatment and different criteria in the selection of patients. The method for determining PRA and the interpretation of these data vary considerably indicating the need for international standardization In the present and in most other similar investigations the criteria for low and normal PRA have been chosen arbitrarily

In essential hypertension a raised sodium and water content in the vascular wall might be of pathogenic importance by increasing the peripheral resistance (14 25) or the sensitivity to catecholamines (7 20) Several studies indicate that diuretics in long-term treatment may reduce the sodium and water content in the vascular wall and thereby decrease the peripheral vascular resistance (16 4) Since the diuretic response to spironolactone is comparable to that of hydrochlorothiazide (17) the former drug might be a useful alternative alone or in combination with thiazide for counteracting side-effects such as hypokalemia or to restore body kalium deficiency (10)

In 6 patients in the normal PRA group the PRA rose 0.6–1 6 ng ang.I/ml/h two hours after furosemide orally whereas a minor or no increase in PRA occurred in the remainder of this group In the former patients the average decrease in mean BP after S_4MD (39 7 mmHg) was significantly greater ($p<0.01$) than in the other patients in this group (26.8 mmHg) MD is known to decrease PRA levels whereas thiazides as well as spironolactone increase them (1 17 18). Although the number of patients is too small to allow any conclusions this finding could indicate that combined treatment with methyldopa and spironolactone is more useful in hypertensive patients whose PRA responds to furosemide

It is well known that diuretics of the thiazide group can induce serious metabolic side-effects, such as reduced glucose tolerance and increased serum uric acid levels when used for long periods. Whether these drugs are liable to influence the serum lipid pattern and contribute to the development of atherosclerosis is a matter of discussion (3 13) Such metabolic side-effects have not been reported during treatment with spironolactone in the doses used in this investigation A significant increase in serum urea was, however observed in our patients after spironolactone indicating that kidney function might have been impaired More serious side-effects such as gynecomastia impotence and menstrual disorders were not observed in the present investigation but suggest, when present that spironolactone may interfere with basic endocrine regulations. It should be observed that a direct inhibitory effect of spironolactone on the adrenal glands has recently been demonstrated (23)

In our low PRA group two of the patients suffered from coronary heart disease and one of them developed cerebral stroke with hemiplegia after this study had been completed whereas similar vascular complications were not observed in the normal PRA group Although the number of patients is small and the observation time short, our data do not support the concept of Brunner et al (5) that low PRA affords protection against serious vascular complications

We conclude that diuretics of the thiazide type as well as spironolactone are useful and generally indispensable alternatives as basic therapy in hypertensive disease More needs to be known about the effects and side-effects in the long term treatment of subjectively healthy people with clinically diagnosed hypertension.

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PLASMA RENIN ACTIVITY BLOOD PRESSURE AND SODIUM EXCRETION DURING TREATMENT WITH CLONIDINE

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Abstract Clonidine 225 µg a day has been given orally for 3 months to 15 patients with essential hypertension. Mean BP was reduced from 159/107 to 143/87 mmHg. The antihypertensive effect of the drug was dissociated from changes in plasma renin activity (PRA) during clonidine treatment. PRA levels decreased initially in 6 patients with the highest PRA values before treatment, but then increased again. In 6 patients with lower pretreatment PRA levels, PRA rose continuously. Opposite patterns of 24-hour urinary sodium excretion were observed in these arbitrary subgroups. The antihypertensive effect of clonidine in essential hypertension appears to be independent of changes in PRA.

Recent observations that clonidine an antihypertensive drug, decreases plasma renin activity (PRA) when administered i.v. (4 9) or orally to normo- and hypertensive people (2, 7 9) have prompted suggestions that suppression of renin secretion may contribute to the antihypertensive effect of clonidine (2 9) The mechanisms by which clonidine exerts its antihypertensive and renin suppressive effects (9) are incompletely understood There is evidence for a suppressive effect on central sympathetic vasomotor mechanisms and peripheral effects as well (4 8)

Previous work on PRA during clonidine therapy has been largely concerned with acute and short time studies (2 4 9). Chronic treatment with clonidine reportedly causes suppression of PRA levels in hypertensive patients (7) In patients with essential hypertension, clonidine reported to cause retention of sodium (? 9) and suppression of urinary aldosterone excretion (2)

We investigated the effect of low oral dose of clonidine on PRA levels BP and urinary excretion of sodium and potassium in patients with mild, essential hypertension The results indicate that suppression of PRA is not a consistent feature during prolonged antihypertensive therapy with clonidine

PATIENTS AND METHODS

Fifteen patients, 3 of them female, mean age 38 7 years (range 21–55), were included in the study Excluded were patients with any of the following signs of secondary hypertension heart failure, renal or hepatic disease metabolic or endocrine diseases, psychiatric disorders known duration of hypertension above 10 years. All had diastolic BP consistently ≥100 mmHg, measured in the supine sitting and upright postures on several occasions during at least 3 months. Individual clinical data are given in Table I Only WHO stage I–II hypertension was included The examination included X-ray of the chest, i.v. pyelography ECG estimation of Na, K, chlorides, and creatinine concentrations in serum, and urinary excretion of Na and K (flame photometry).

Study protocol. A detailed information sheet was given to each patient. Eleven patients had no previous antihypertensive treatment. The other 4 were without drugs for at least 2 weeks before starting the study Clonidine, 75 µg 3 times day (Catapresan Boehringer Ingelheim) was given orally for 3 months on non-blind, outpatient basis No dietary restriction was introduced, and ordinary outpatient antihypertensive treatment was mimicked as closely as possible Each patient was seen immediately before treatment, after 1 month and after 3 months Each visit to the clinic included clinical examination, BP measurements, and collection of samples for the estimation of PRA serum concentration of Na and K, and urinary excretion of Na and K.

During the course of the study the physicians examining patients were not aware of PRA data and the laboratory personnel did not know about the clinical data

Renin assay Venous blood for PRA assay was collected into ice-chilled tubes containing disodium-EDTA (15 mM final conc) after 12 hours of fasting and 2 hours of upright posture (walking) at 10–11 a.m. Plasma, separated by centrifugation at +4°C was stored at −20°C until assayed. PRA was estimated with radioimmunoassay for

Table I *Clinical data of the patients studied*
LVH = left ventricular hypertrophy

Pat no	Age (y)	Duration of hypertension (y)	Supine BP 1st visit, without drugs (mmHg)	Serum creatinine (µmole/l)	Possible complications of hypertension
1	34	3.5	160/100	91	Subarachnoidal haemorrhage in 1972
2	25	6	160/100	88	None
3	41	0.5	165/105	101	None
4	33	1	160/100	86	None
5	44	0.5	155/100	91	None
6	40	0.5	145/100	82	None
7	55	0.5	150/100	85	Slight signs of LVH
8*	43	0.5	170/110	76	None
9*	35	3.5	180/120	97	Slight signs of LVH
10	40	0 5	170/110	82	None
11	41	0.5	160/110	113	None
12	21	0.5	190/110	84	None
13	48	1	170/115	96	Slight signs of LVH
14	33	0.5	180/120	100	None
15	28	2	160/100	85	None
Mean ±S E.M	38 7 ±2.6	1 4 ±0 43	165±3 1 107±1 9	90±2.5	

Female

angiotensin I by a method modified according to the method of Stockigt et al (10) Reference values for 23 normotensive healthy subjects under identical conditions were 1 06±0 13 (S E.) ng/ml/h. Interassay variation was 11% (S D) and intraassay variation 8.2% Significant correlations were found between data obtained from identical plasma samples with the present radioimmunoassay and PRA bioassay according t Boucher et al (3) ($n=16$ $=0$ 77)

RESULTS

asma renin activity After 1 month of clonidine treatment mean PRA decreased from 0.81 to 0 61 ng/ml/h (not significant) and then increased to 1 00 ng/ml/h ($p<0$ 05) after 2 further months (Table II) Two patterns of change in PRA could be distinguished namely a decrease of the highest levels followed by an increase after 3 months (Fig 1) and an increase of PRA in those with pretreatment values below the mean followed by either a further increase or decreases with further months of clonidine therapy

A study of the 6 patients with the highest pretreatment PRA levels and the 6 with low pretreatment and continuously increasing PRA values (Fig. 1) revealed a contrary pattern for sodium excretion.

Blood pressure BP decreased significantly after one month of clonidine therapy (systolic supine and upright $p<0$ 001 diastolic $p<0$ 001) but did not decrease significantly with 2 further months of drug treatment (Table II) Two of 15 patients had end

Table II *Blood pressure plasma renin activity and urinary 24-hour excretion of sodium and potassium (mean*±S E.)

	BP (mmHg) Supine Systolic	BP Supine Diastolic	BP Upright Systolic	BP Upright Diastolic	PRA (ng/ml/h)	Urinary 24-hour excretion (mEq) Na	K
Pretreatment	158±3.6	106±2 3	159±3.8	107±2 6	0.81±0 22	209±18	78±6 3
After 1 month	143±3 4**	92±2.2**	143±2.5	93±2.5**	0 61±0.11	185±13	75±6 0
After 3 months	145±3 3	89±2.0	143±3.2	87±1 9	1 00±0 15	204±20	75±5 7

$p<0$ 05 $p<0.001$

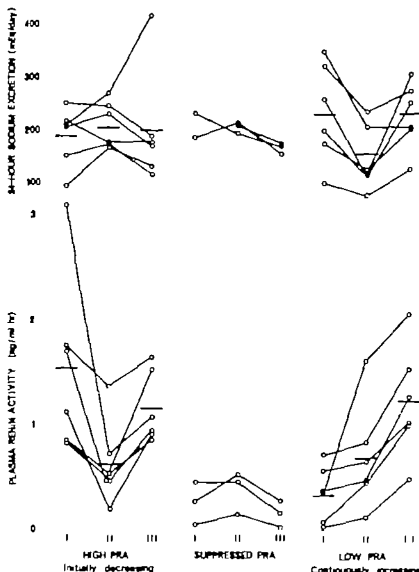


Fig. 1. PRA and 24-hour urinary excretion of sodium before treatment (*I*), after 1 month (*II*), and after 3 months (*III*) of clonidine therapy. Mean values are indicated by horizontal lines.

treatment diastolic BP ≥100 mmHg and were considered clinical failures. The antihypertensive effect of clonidine was considered good and the side effects observed were few (Table III). Although the best responses of diastolic BP were observed in patients with low pretreatment PRA levels (Fig. 2) no significant correlation was found between diastolic BP decrease and either pretreatment ($N=15$, $r=0.37$) or end-treatment PRA values ($N=15$, $r=0.14$).

Excretion of sodium and potassium. The mean pretreatment 24-hour sodium excretion decreased after 1 month of clonidine therapy (not significant, Table II) and returned to pretreatment levels after 3 months of drug treatment. In 5 of the 6 patients with the highest pretreatment PRA levels (Fig. 1), an increase in sodium excretion after 1 month (not significant) was followed by decreased 24-hour sodium excretion. In the 6 patients with low PRA levels before treatment and continuously increasing PRA during clonidine therapy, a significant decrease ($p<0.05$) in sodium excretion was followed by an increase ($p<0.05$) back to pretreatment levels.

Table III *Antihypertensive effect of clonidine*

Duration of treatment (mo)	No. of pats.	Normotensive[a]	Responsive[b]	Side-effects (no. of pats)
1	15	6	5	Drowsiness 5 Dry mouth 3 Swollen legs 2
3	15	8	5	Drowsiness 1

[a] BP≤140/90 mmHg (upright).
[b] BP≤150/95 mmHg (upright) or BP reduced 20/20 mmHg or more.

Urinary 24-hour excretion of potassium and serum concentrations of sodium and potassium, did not change significantly during the study (Table II)

DISCUSSION

The present study in contrast to previous reports (2 7 9) revealed increased PRA levels during clonidine therapy in a considerable proportion of patients with mild essential hypertension. Decreased PRA values were observed in patients with the highest pretreatment PRA levels but even in these patients PRA levels were not significantly suppressed after 2 further months of clonidine therapy Much of the differences between these results and previous reports may depend on the low dose of clonidine (225 μg a day) used by us. Moreover previous investigations on the effect of clonidine on PRA levels have been largely concerned with acute (4 9) or short-time (2 9) effects, while we studied the effect of clonidine during 3 months Previous reports (4 7 9) on PRA during clonidine administration have been based upon the bioassay method of Boucher et al (3) or radioimmunoassay of angiotensin I (2) Data obtained by our immunoassay method (10) correlated significantly with results using bioassay according to Boucher et al Therefore it appears unlikely that differences in results could be explained by different renin assay techniques

Baer et al (2) reported suppression of PRA in 22 hypertensive patients on a constant metabolic regimen and treated with 300 μg clonidine a day for 5 days They also observed sodium retention and suppression of urinary aldosterone excretion. They suggested that clonidine may be useful for patients with hyperreninemia and aldosteronism Our data do not favour such a conclusion Hökfelt et al (7) reported suppression of PRA levels in various types of hypertension with clonidine doses ranging from 300 to 900 μg daily for 6–30 days. However clonidine did not inhibit an increase in PRA in response to sodium restriction in one of their patients

Clonidine reportedly causes a decrease in renal vascular resistance in patients with essential hypertension during orthostatic stimulation with 45° tilting (9) We measured PRA after stimulation in upright posture The increased levels of PRA during treatment with clonidine reported here may be due to decreased renal vascular resistance Other sympathetic inhibitors (reserpine trimetaphan) reported to increase PRA in dogs with experimental hypertension (1) may cause release of renin by a similar mechanism

During dietary sodium restriction (30 mEq Na daily) a powerful stimulus for renin release clonidine 100 μg 4 times a day administered orally for 5 days to patients with essential hypertension caused suppression of PRA in both the supine and in the 45 tilted posture (9) This may be explained in part by initial retention of sodium and fluid caused by clonidine (2 9) in addition to inhibition of sympathetic nervous mechanisms (8). In agreement with such an explanation we observed the most impressive reductions of PRA in the patients with ankle edema and weight gain. During sodium repletion, however when PRA levels are generally lower the inhibitory effect of clonidine on renal reflex vasoconstriction in response to orthostatic stimulation (9) may override the inhibitory sympathetic

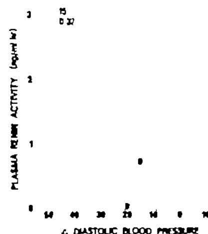


Fig 2 PRA before treatment compared to change of diastolic BP in upright posture. Linear regression analysis not significant.

nervous mechanisms (8) The net effect would be an increase in PRA during upright posture as observed by us in low renin hypertensive patients

The antihypertensive effect of the low dose of clonidine (225 μg daily) used in this study appeared better than that reported by Onesti et al. (9) using 400–900 μg a day The lower dose of clonidine may explain the lower frequency of side-effects observed by us Antihypertensive responses similar to those reported here were observed by Baer et al (2) in patients receiving 300 μg clonidine daily for 5 days.

The antihypertensive effect of clonidine was not significantly correlated to pretreatment PRA levels and was dissociated from changes in PRA levels during therapy This was not altered when our patients were divided into subgroups according to 24-hour urinary excretion of sodium as described by Brunner et al (5) It would appear that clonidine may be used as an antihypertensive agent even in mild, essential hypertension, regardless of the PRA levels

The possibility remains that in clinical states with inappropriate hypersecretion of renin, e.g. renovascular hypertension, suppression of renin release may be beneficial However this may require higher doses as used by Hökfelt et al (7) Moreover the hypertensive and vasoconstricting effect of exogenous angiotensin and catecholamines in rats is blunted by clonidine (6) Thus clonidine may in fact stimulate renin release while simultaneously counteracting the pressor activity of angiotensin generated by renin.

The opposite patterns of sodium excretion observed in patients with "high" and low continuously increasing PRA levels (Fig. 1) seem to indicate that clonidine causes different changes in sodium excretion and perhaps ingestion in these two arbitrary subgroups of essential hypertension The tendency towards stabilization of sodium excretion after 3 months of clonidine therapy suggests that the effect on sodium balance is transient followed by adaptive adjustments which do not abolish the antihypertensive effect of the drug.

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GLUCOSE–INSULIN TREATMENT OF LACTIC ACIDOSIS IN PHENFORMIN TREATED DIABETICS

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Abstract. Four cases of lactic acidosis in phenformin-treated diabetics are presented. Blood lactate before treatment was 5 7 9 4 10.7 and 17 8 mM/l respectively. Treatment with glucose Insulin and bicarbonate resulted in correction of acidosis and hyperlactataemia. This therapy is recommended in phenformin-induced lactic acidosis.

Lactic acidosis in phenformin-treated diabetics has been described since 1959 (19). This type of lactic acidosis is a serious condition, characterized biochemically by accumulation of lactic acid causing metabolic acidosis, and clinically by anorexia vomiting, and impairment of consciousness varying from lethargy to coma. Various therapeutic principles have been employed intensive infusion of bicarbonate (1 13) peritoneal dialysis (15), and hemodialysis (17) but mortality has remained high, approximately 50% (5)

Since Johnson and Waterhouse in 1968 (9) presented a case of phenformin-induced lactic acidosis in which glucose–insulin treatment had a remarkable effect, a total of 7 patients surviving after this therapy has been reported (8 13 16 18). This is a report of 4 additional cases occurring in May–Oct 1974 all successfully treated with glucose insulin and bicarbonate

CASE REPORTS

Case 1

A 59-year-old woman who had had mild diabetes for 1 years. One year before admission, treatment of her diabetes was changed from carbutamide to phenformin 100 mg, and glibenclamide 5 mg daily During the last weeks before admission she had lost her appetite On the day before admission she had suffered from abdominal pai and vomiting On admission she appeared pale and drowsy Pulse rate was 104/min, BP 145/85 mmHg rectal temperature 36°C Blood chemistry showed an increased "anion gap" ((Na +K)–(Cl⁻+HCO_3^-)>20 mEq/l), acidosis, nd hypoglycaemia (Table I) indicating lactic acidosis. Treatment consisted of injection of 50 ml 50% glucose+16 IU soluble insulin, repeated after 3 hours. In addition she received 83 mEq bicarbonate and 1 500 ml 5% glucose Twelve hours after initiation of this therapy her clinical condition had improved and drowsiness had disappeared Treatment of her diabetes was changed to insulin NPH and she was discharged in good health.

Cas 2

A 85-year-old woman w th diabetes of 10 years duration years, treated with phenformin, 50 mg daily Fi years prior to admission arterial hypertension was diagnosed and treatment with methyldopa was started. One month before admission she began to lose weight owing to anorexia and frequent vomiting. On the last day before admission she had been confused and had according to her relatives taken overdoses of phenformin and methyldopa. *On admission she was pale, somnolent,* confused, and had Kussmaul respirations. BP was 140/80 mmHg decreasing to 100/60 Laboratory data showed metabolic acidosis hyperlactataemia (Table I), and Hb 8.9 g/100 ml. The condition was treated with 50 ml 50% glucose+20 IU soluble insulin njected i twice, with an interval of 4 hours. In addition 240 mEq bicarbonate and 500 ml blood were given After 5 hours, acidosi had been corrected but blood lactat was still 8.6 mM/l (Fig 1) During the following 8 hours she received 2 000 ml 5% glucose+20 IU soluble insulin In the same period her clinical condition improved and she became rational At discharge a satisfactory regulation of her diabetes had been achieved th just diet.

Cas 3

A 85-year-old woman with diabetes of 10 years duration, treated with phenformin, 100 mg daily was admitted for treatment of arterial hypertension and epistaxis Ow ng t moderate arteriosclerotic heart disease she received digoxin and furosemide After admission medications were continued in unaltered doses The epistaxis had been stopped by an anterior packing which was removed after 6 days Tw days later she lost appetite and began to vomit

Table I *Laboratory data before treatment with glucose insulin and bicarbonate*

	Case no			
	1	2	3	4
Blood lactate (mM/l)	9 4	10.7	5 7	17.8
Plasma glucose (mg/100 ml)	46	209	161	161
Plasma bicarbonate (mEq/l)	11	12	16	5
Plasma chloride (mEq/l)	102	–	88	–
Plasma sodium (mEq/l)	142	136	131	145
Plasma potassium (mEq/l)	5.8	4.0	4.6	5.3
Plasma creatinine (μM/l)	155	305	265	190
Blood pH	7 17	7.30	7.29	6.79
Blood pCO_2 (mmHg)	27	18	27	19
Blood O_2-sat. (%)	92	97	94	87
Blood base-excess (mEq/l)	−17.5	−15 7	−12.3	−18.7
Urine glucose	0	0	0	+
Urine ketone	+	+	0	++
Urine pH	6	5	–	–

She was pale and hyperventilating. An increased anion gap and a metabolic acidosis suggested lactic acidosis, verified by determination of blood lactate (Table I). She was treated with 90 ml 50% glucose+16 IU soluble insulin i. repeated after 4 hours, 166 mEq bicarbonate and during the following days with insulin NPH and continuous infusion of 5% glucose After an initial rise, blood lactate decreased, and normal values were reached after hours (Fig 1) The course thereafter was uneventful left hospital with a diabetes regimen consisting of diet y

Case 4

A 74-year-old diabetic woman, treated with insulin for 14 years was admitted because of vertigo After admission regulation of her diabetes was found unsatisfactory and treatment with diet and phenformin was instituted. After 10 days, the dose of phenformin was increased from 50 to 75 mg daily and 8 IU insulin NPH was added Preceded by anorexia and drowsiness, the patient was found one morning 15 days later deeply comatose Her respirations were rapid and deep the pulse was 120/min, BP 180/90 mmHg and temperature 37 1°C decreasing to 35 1 An i. drip containing Ringer lactate was started. Owing to the finding of normoglycaemia and a pronounced metabolic acidosis (Table I) lactic acidosis was suspected. Ringer lactate was discontinued after 5 hours, and blood samples showed blood lactate 18 7 mM/l At this time she had received 500 ml Ringer lactate (14 mM lactate) a dose insufficient to explain the marked elevation of blood lactate. Treatment consisted of i v injection of 50 ml 50% glucose+1 IU soluble insulin repeated after 5 hours, 750 mEq bicarbonate and 1000 ml 5% glucose+24 IU soluble insulin given over 4 hours. Twenty-four hours after institution of this therapy arterial pH was 7 46 and the patient was awake During the remainder of the hospitalization her diabetes was well controlled with diet and insulin.

DISCUSSION

Biochemical changes in phenformin-induced lactic acidosis consist of an accumulation of lactic acid reflected in an increased anion gap leading to metabolic acidosis. Ketosis is absent or moderate hypoglycaemia is frequently present (1) Lactate is formed from pyruvate and is an end product in anaerobic glucolysis Lactate is eliminated only after reconversion to pyruvate which in turn is partly oxidized in Krebs cycle partly utilized in gluconeogenesis (11)

The exact mode of action of phenformin is unknown Based on animal in vitro investigations and on some human experiments several mechanisms have been suggested However their relative contributions in the diabetic human organism are not clear (5) These mechanisms are: increased glucolysis (7 10), possibly due to an inhibition of mitochondrial respiration (3 7) reduced gluconeogenesis (4 7 14) increased muscular glucose uptake (2, 7) and decreased intestinal glucose absorption (6 12) These primary actions of phenformin tend to induce a secondary reduction of glucose level and under special circumstances a marked increase in lactate level resulting in acidosis

Temporarily or persistently impaired renal function which was found in all four cases is consid-

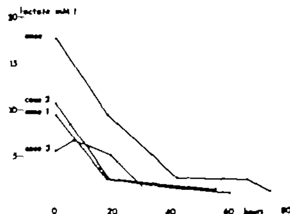


Fig 1 Blood lactate during treatment with glucose insulin and bicarbonate

ered an important pathogenetic factor probably due to reduced elimination of phenformin (5 13) Treatment of lactic acidosis with bicarbonate or dialysis has mainly been directed towards a correction of this acidosis. It is doubtful however whether the primary disturbances in glucolysis and gluconeogenesis are affected. The action of glucose–insulin in lactic acidosis is unknown. Johnson and Waterhouse (9) suggested that a block in pyruvate utilization was relieved However it seems important to attempt to maintain a normal intracellular glucose metabolism through administration of glucose and insulin.

Considering the high mortality in phenformin-induced lactic acidosis and the favourable experience with a therapy consisting of glucose insulin and bicarbonate this treatment as outlined in the present study is recommended.

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DIPPING PROCEDURE FOR BLOOD GLUCOSE DETERMINATION WITH DEXTROSTIX AND THE EYETONE REFLECTANCF METER

Assessment of a Practical Technique

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Abstract A dipping procedure for blood glucose determination with the Dextrostix Ey tone system has been evaluated. The procedure involves the immersion of the Dextrosti reagent area for 1 min in tube of whole blood followed by wash, blotting and reading as in the regular procedure. Sixty-f ve blood samples covering wide glucose concentration range were estimated for thei glucose content in random order both by the dipping procedure and conventional Dextrostix Eyetone procedure. An almost perfect agreement between the two methods w found the coefficient of correlation being 0 99 and the regression line ery close to the ideal line The presence of Dextrosti reagent area in the blood was found to bring about glycolysis. Except at high blood glucose levels, this glycolysis, however was insignificant if the trip was correctly removed after 1 min. The dipping procedure overcomes the main technical problem of conventional procedures the inconsistency of the drop application on the reagent area A it is easy to perform and a reliable alternative to conventional procedures it is recommendable in all cases where blood samples are available.

In a recent Scandinavian multicentre study blood glucose determination with the new Dextrostix reflectance meter Eyetone, was found to be a quick and very reliable alternative to conventional laboratory methods (4) However this new alternative still poses technical problem using conventional techniques it is often difficult to get the complete reagent surface of the strip covered with blood within sufficiently few seconds to ensure exact timing of the procedure n addition, the amount of blood applied may influence the result (1 2)

For this reason an alternate procedure for Dextrostix, in which the strip is immersed in a sample of whole blood obtained from enipunct re has been introduced in our laboratory This report concerns a comparison of the new method with the current one.

MATERIAL AND METHODS

Samples of enous blood were taken in heparinized tubes from both non-diabetics and diabetics Hypoglycemic specimens were also collected from patients submitted to an insulin-hypoglycemia test for other reasons, and hyperglycemic specimens were also prepared by adding concentrated aqueous glucose solution. A total of 65 samples were investigated in triplicate and random order by both of the following procedures:

The regular procedu according to the operating manual for handling the Dextrosti strip and the Eyetone instrument Blood was applied to the eagent area of the strip by means of a pipett The Eyetone instrument was supplied by Dr A. Wagner Ames Co. Scandinavia

The d pping procedur 1) The end of the Dextrostix strip opposite the reagent area was bent. 2) The Dextrostix was then inserted into the tube of well mixed blood the bent end being allowed to hook onto the rim of the tube and thus prevent the strip from falling in 3) The Dextrosti was left in the blood for xactly 60 sec 4) Thereafter the strip was removed washed blotted and read as in the regular procedure

The eagent area of the Dextrosti strip is covered with semipermeable membrane which permits the glycolytic enzymes the area to escape into the surrounding blood Consequently the blood glucose concentration in the sample might decrease during the dipping procedure and thus nvalidate control readings on the same sample To evaluate the importance of this induced glycolysis, the blood glucose oncentration was determined by means of an AutoAnalyzer (Hexokinase-procedure (5)) in each of three samples containing various amounts of glucose on each sample (3 ml) sixfold estimations were carried out before and 1 ., and 3 min after the insertion of Dextrosti strip into the sample

Calculations

Orthogonal regression analysis was applied to test the correlation between blood glucose determination with the tw methods, and analysi of variance was used to estimate their precision The significance of differences between variances was established by means of the *F* test, and differences between means by Student *t*-test. P alues les than 0 05 were considered

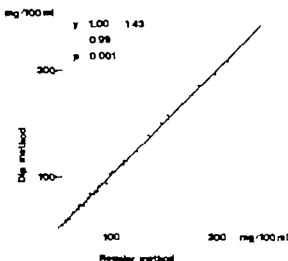


Fig. 1 Correlation between the regular and the dipping procedure for blood glucose determination with the Dextrost Eyetone system. Each point is the mean of corresponding triplicate measu ements.

RESULTS

Correlation bet een the two Dext ostix–Eyetone pr ocedures

In Fig. 1 the correspond'ng mean values of the two methods are plotted against each other. The coefficient of correlation is 0.99 and the regression line =1.00x+1.43 ery close to the ideal line. This ind'cates a high degree of correlation between the regular method and the proposed alternative

ion and arian e

Summary of the precision and ariance data is en n Tables I and II respectively. Both the means and the S.D. of the two methods are quite similar. Moreover the variance of the two methods is not significantly different at any level of blood glucose within the working range of the Eyetone instrument.

Escape f glycolyti en mes from the Dextrostix into s rround ng blood sampl

Immersion of a Dextrostix trip for 3 min in 3 ml blood induced a significant fall in the glucose le el of each of three samples investigated (Fig. 2). However immersion for 1 min significantly suppressed the glucose level only in the sample with the highest glucose content.

Table I *Precision of the regula and the dipping method*

	Regular method	Dipping method
Mean	130.0	131.3
S.D.	4.8	4.7
Coefficient of variation	3.7	3.6

Table II *Variance of the regular and the dipping method at different levels of blood glucose*

Range (mg/100 ml)	No of deter minations	Variance Regular method	Variance Dipping method	Comparison of variances
10– 50	15	.60	4.60	n.s.
51 100	1	6 17	7 40	n.s.
101 150	7	11 95	5 86	n.s
151 200	4	29 17	32.83	n.s.
201–250	6	66.33	31.22	n.s.
251 300	5	43.33	66.67	n.s.
301–350	6	68 06	48.61	n.s.
351–400	1	100.00	100 00	s.
10–400	65	2.80	22.29	n

n.s. =no significant difference

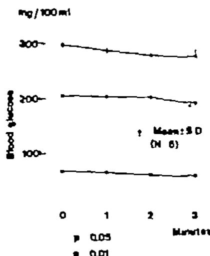


Fig. 2 Influence of immersion of the Dextrostix trip for 1–3 min on the blood glucose level of the sample. Δ=significant suppression of blood glucose concentration.

DISCUSSION

The results indicate almost perfect agreement between the dipping and the conventional Dextrostix–Eyetone procedures. In the latter case the blood was applied with a pipette in order to cover the reagent area completely within a few seconds. This ensures exact timing, which is necessary to obtain precise results (2). In other words, the dipping procedure was tested against the best of the current procedures.

The applicability of the dipping procedure is limited by the need to have a fresh venous blood sample. However, as the Eyetone instrument is as accurate for all practical purposes as the automatized apparatuses in general use (4), it may gain access to fields (e.g. hospitals) where blood samples taken for other reasons are often available. In such cases the dipping procedure is a reliable alternative to the current finger-prick or earlobe-prick procedures. It is simpler to perform than any of the current procedures, including application by a pipette. Furthermore, it completely overcomes the inconsistency of the drop application in covering the complete reagent surface of the Dextrostix.

The glycolysis in the sample induced by the immersed Dextrostix strip was insignificant, except at high glucose levels, provided the strip was in fact removed after 60 sec. And anyhow, this glycolysis is of little practical importance because control readings of blood glucose concentration on the same sample are not indicated as a rule. Another problem is the spontaneous glycolysis in the sample under study, which necessitates storage of the sample at 4°C if not analysed immediately (3).

Bearing in mind these small limitations, it may be concluded that the dipping procedure for Dextrostix is a reliable alternative to conventional methods and can be recommended whenever whole blood samples are available for other reasons.

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SUBCUTANEOUS ADMINISTRATION OF SODIUM L THYROXINE TO PATIENTS WITH HYPOTHYROIDISM

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Abstract Tw patients with primary hypothyroidism are described in whom an euthyroid condition could not be achieved by oral administration of l-thyroxine, l-triiodothyronine or desiccated thyroid Daily subcutaneous injections of l-thyroxine normalized the thyroxine and *triiodothyronine level n the blood and the patients be-*came euthyroid

Treatment of patients with hypothyroidism is usually no problem since oral administration of l-thyroxine l-triiodothyronine or desiccated thyroid extract corrects the symptoms and signs of hypothyroidism Exceptions to this are extremely rare even for a thyroid clinic with a large number of thyroid patients

This report describes two patients with hypothyroidism in whom the hypometabolic state could not be corrected by oral administration of thyroid hormones. An euthyroid condition was achieved with daily subcutaneous injections of l-thyroxine

MATERIAL AND METHODS

Subject The first patient wa a 50-year-old house wif who developed hypothyroidism after subtotal thyroidectomy for an at xic nodular goiter She developed typical clinical nd laboratory signs of hypothyroidism hich could not be corrected by oral administration of thyroid hormones. She was nder observation several times in the ward and we have no doubt that she took her *pills* The replacement dose was increased but even with 0 4 mg l-thyroxine plu 0 06 mg l-triiodothyronine no improvement was observed in the patient pronounced clinical and laboratory signs of hypothyroidism

The second patient was 72-year-old single woman who for several years had been successfully treated with desiccated thyroid xtract for primary hypothyroidism caused by Hashimoto thyroiditis. The firm that produced her desiccated thyroid pills had stopped manufacturing them some years previously the patient had bought large stock of pill just before production was terminated and she was clinically euthyroid The laboratory tests were *within the normal range while she took her pills and the* problem started when her doctor tried other thyroid tablets. She was subsequently referred to ou hospital for investigation and the results demonstrated that no other thyroid pills could be sed She experienced severe abdominal discomfort about an hour after taking the new drugs. We tested all available drugs containing l-thyroxine l-triiodothyronine or desiccated thyroid.

Laboratory tests. The serum concentrations of thyro ine and triiodothyronine were determined by a radioimmunoassay technique (4). The normal range for thyroxine is 6 9±1 3 μg/100 ml (mean±S D.) and for triiodothyronine 115±22 ng/100 ml (mean±S.D). TSH w s measured with commercial kit based upon radioimmunoassay technique

l-thyroxine for parenteral administration wa prepared from stock solution containing the sodium salt of l-thyroxine (1 0 mg), sodium derivates of formaldehyde sulphoxylate (2.0 mg) and sodium salt of amino acetic acid (4 1 mg) sodium hydroxide (1 8 mg) and sterile water ad 1 0 ml The final concentration of l-thyroxine was 0 4 mg/ml at pH 10.5

RESULTS

Parenteral administration of l-thyroxine was initiated when it became obvious that further oral administration of l thyroxine l-triiodothyronine or *desiccated thyroid extract would have no therapeu-*tic effect. The instruction to the patients regarding the subcutaneous adm nistration was given in the same way as to patients with diabetes mellitus on insulin therapy

Daily subcutaneous injections of 0...–0 3 mg l-thyroxine completely reversed the hypothyroid condition Both patients were clinically euthyroid after two month treatment The serum levels of

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thyroxine was 9 6 and 14.2 μg/100 ml and of triiodothyronine 108 and 100 ng/100 ml in the two patients TSH was within the normal range No untoward reactions have as yet been seen since the initiation of therapy

DISCUSSION

The results demonstrate that an euthyroid condition can be achieved by daily subcutaneous injections of l-thyroxine to hypothyroid patients. The patients can give themselves the injections without any problems

I v administration of thyroid hormones mainly l-triiodothyronine has been described for the treatment of myxoedema coma (1 3 5 6). However no previous studies seem to have been published concerning the long-term treatment of hypothyroidism with subcutaneous administration of thyroid hormones Also these patients have been followed up with respect to dose and hormone level in the blood

The underlying reason for the untoward reaction to oral administration of the thyroid hormones is not fully understood It is possible that the first patient has a defective absorption of thyroid hormones in the gastrointestinal tract It is also conceivable that the abdominal discomfort experienced by the second patient is an allergic manifestation which in all probability is not caused by the thyroid hormones as such but possibly by another factor present in the tablets

This investigation also verifies that thyroxine is converted to triiodothyronine in the body as a normal level of triiodothyronine was found after the administration of thyroxine The tissue responsible for this conversion is not yet known but this investigation seems to exclude the gastrointestinal tract as a possible site

The concentration of thyroxine was above the normal range despite a normal triiodothyronine level and an euthyroid condition This is seen in patients maintained on l-thyroxine and is probably caused by the absence of the triiodothyronine formed by the thyroid gland

ACKNOWLEDGEMENTS

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THE SMOKING HABITS OF MEN WITH INTERMITTENT CLAUDICATION

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Abstract Smoking habits among 54 male patients with intermittent claudication (IC) and 200 healthy 50-year-old men from the same county have been studied. The prevalence of smokers at the age of 50 was 98% among the IC patients against 46% among the healthy controls The percentage of heavy smokers and the total tobacco consumption were not significantly different in the two groups. However the percentage of smokers who began to smoke before the age of 15 was significantly higher in IC patients than in the healthy group, 28% to 7%

Many studies have shown that the majority of patients suffering from intermittent claudication (IC) are smokers The frequency in these studies often approaches 100% (3 6 9 10)

When the prevalence of smoking among patients with IC is discussed reference should be made to the prevalence in the general community The frequency of smoking among middle-aged men is high in many countries 70% i the USA and England 69% in a Swiss population (12) 78% i a Danish study (5) and 70% in a Norwegian study (4) In Sweden the figures are lower 50% in Uppsala (7) 62% in Malmo (8).

Few of the earlier studies include a control group The results of a comparison of the smoking habits of men with and without IC from a restricted geographical area are reported here against the background of this regional variation The results are related to earlier studies.

MATERIAL AND METHODS

Free health xaminations have been offered to all men Uppsala born in 1920–24 The main object of the examination was to clarify and treat cardiovascular risk factors Two hundred men born in 1923 were selected at random among these subjects to compare their smoking habits with those of group of 54 patients with IC

A total of 170 patient with IC (132 men 38 women) were investigated at the Geriatric Clinic in Uppsala between May 1971 and June 1973 the majority however being referred from other counties or very old. Among these 54 male IC patients born after 1903 from the county of Uppsala were selected for study of their smoking habits The selection was performed to make the patient and control groups as comparabl as possible with regard to place of domicile and age

A detailed history of smoking habits was obtained at the health control described above Smoking habits were clarified from the age at which the subjects began to smoke up to the time of examination. Changes in habit were specially examined. The same questionnaire was sed in telephone interviews with IC patients. A small group of patients who could not be contacted by telephone were questioned by letter and replies were obtained from 100% of IC patients.

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RESULTS

Fig. 1 shows the age distribution (mean 61 0 median 62.5 years) and number of smokers in the IC group The number of IC patients who had smoked 5 years before the study is also shown. Forty-one patients (76%) were smokers in 1973 while 52 (96%) had smoked in 1968 Only one patient stated that he had never smoked Thi man suffered from diabetes mellitus hypertension and hyperlipidaemia. One patient smoked between the age of 12 and 50 but stopped before 1968 Of the 200 healthy men born in 1923 9? (46%) stated that they smoked at the time of the xamination in 1973 while 112 (56%) had smoked 5 years earlier Sixty-one men (31%) stated that they had never smoked

Smoking habits between 1968 and 1973 were examined in an effort to illustrate the effect on them of disease. (It was found that m majority of the



Fig 1 Age distribution and number of smokers of 54 me with intermittent claud cation in 1968 and 1973

patients their disease had begun between these years) Eleven (21 %) of the IC patients stopped smoking compared with 20 (18 %) controls A further 1? (23 %) of the smoking IC patients and 5 (4 %) of the smoking controls reduced their tobacco consumption

As a large number of IC patients had altered their smoking habits the daily tobacco consumption in 1968 was estimated As shown in Table I there were no obvious differences between the two groups Thus there was no overrepresentation of heavy smokers in the IC material

Total tobacco consumption up to the age of 50 was found to average 140 kg for IC patients and 151 for controls Table II shows the consumption ided into three arbitrary classes The percentage smokers who had consumed more than 250 kg obacco was almost identical in both groups. A higher proportion of the IC patients had consumed

Table I *Tobacco cons mption in 1968 of men with intermittent claudic atio (n=52) and of controls (n=112)*

Relative frequency is gi en for IC patients to the left of the slanting line and for control t the right

Pipe tobacco (g/week)	No of cigarettes/day 0	1 10	11 20	>20
0		19_/23_	25 0/27 7	7 7/6.3
≤50	21_/17.0	15 1/ 7 1	3 8/11 6	–/0 9
>50	5.8/ 5 4	1.9/–		–/0 9

Table II *Total consumption of tobacco up to the age of 50 in 53 men with intermittent claudication and in 139 controls*

Tobacco consumption (kg)	IC patients N	%	Controls N	%
≤100	19	34	62	45
101–250	30	57	61	44
>250	5	9	16	11

101–_50 kg tobacco but this difference is not significant ($\chi^2=2\ 49$ $p>0\ 05$)

The age at which IC patients started to smoke averaged 16 8 years whereas for the controls it was 19 1 years This difference is significant ($p<0\ 005$) A subdivision into three arbitrary groups is pre sented in Table III 28 % of IC patients and 7 % of the smokers in the control group began to smoke before the age of 15 This difference is also significant ($p<0\ 005$). Table IV shows the average age of commencing to smoke and illustrates its variation among IC patients divided into 5-year groups ac cording to year of birth The duration of smoking up to the age of 50 was 32 2±3 4 years (mean ±S D) in the IC patients against _8.5±6 0 years in the controls This difference is significant ($p<0\ 001$).

Table III *Age at which 53 men with intermittent claudication and 139 controls began to smoke*

Age (y)	IC patients N	%	Controls N	%
<15	15	28	10	7
15–19	25	47	79	57
≥20	13	25	50	36

Table IV *Age at which 53 men with intermittent claudication began to smoke cla sified according to year of birth*

	Year of birth 1903–07	1908–12	1913–17	1918–22	1923–
N	16	18	7	8	4
Range	12 20	14–23	11 _5	13–20	1?–25
Mean age (y)	15.5	18.2	16.6	15.8	17 8

DISCUSSION

Of the studies listed in the introduction only that by Juergens et al (9) has any form of control material This was a group of patients from the same clinic aged 25–55 who did not suffer from vascular dis ease Of them 74% were smokers We had the opportunity to compare our patients with healthy 50-year-old men, selected from a population study In view of the possibility of regional variations in the prevalence of smoking, the smoking habits of male IC patients from the county of Uppsala were studied Only IC patients born after 1903 were selected, in an attempt to reduce the age difference between patients and controls while yielding a suitably-sized patient group. The IC patients were also questioned about their smoking habits at the age of 50 and 98% stated that they had smoked at that age against 46% of the controls Thus the present study reveals that even when the IC group is recruited from a population with a relatively low prevalence of smokers, it still contains the high proportion of smokers previously reported Further it should be noted that in 1973 when the IC patients had a mean age of 61 years 76% of them smoked a surprisingly high figure as the prevalence of smoking decreases with age

Smoking has been discussed as a causative factor for cardiovascular disease especially after Astrup (1) showed the importance of the carbon monoxide concentration in the blood for the development of atheromatosis in the vessel wall On the other hand when discussing findings such as the present over representation of smokers among IC patients it must be born in mind that other factors (psycho-social, alcohol intake, etc) may be causative and that smoking just co-varies with these This study was limited to an examination of the smoking habits alone to see if these differed from those of healthy men.

If smoking is a causative factor for IC one would expect to find an overrepresentation of heavy smokers among IC patients Juergens et al (9) found no difference in numbers of heavy smokers among IC patients and controls. We have investigated tobacco consumption both as total consumption up to 50 years of age and as daily consumption during 1968 and found no difference between our two groups The year 1968 was chosen as many IC patients changed their smoking habits after the début of disease between 1968 and 1973

The lack of correlation between number of heavy smokers and IC disease may be due to several factors. Wald et al (11) have shown that the prevalence of vascular disease is better correlated to carboxyhaemoglobin concentration in blood than to tobacco consumption this may be due to the fact that individuals with the same tobacco consumption may inhale to different degrees

Other factors concerning smoking might be of importance. It is noteworthy that the percentage of smokers in our study who began to smoke before the age of 15 is significantly higher among the IC patients than among the controls, 28% to 7% It has been common only in recent decades for young teenagers to start smoking. Table IV shows however that throughout the age range in the IC group a number of patients started smoking before the age of 15 This low age besides involving more years of exposure to carbon monoxide might also mean that more immature and vulnerable tissues are exposed to carbon monoxide It is possible that starting smoking at a young age is a factor of importance for the development of arteriosclerotic vascular disease.

The number of patients who ceased smoking d r ing the investigated 5-year period was not greater than the number of those who stopped spontaneously in the control group All patients in the IC group had been given active anti-smoking advice Burt et al (?) reported that up to 60% of smokers who suffer a heart attack and are actively advised to stop smoking are non-smokers one year afterwards and the same tendency has been found in a local material (to be published). This may of course be due to the fact that a heart attack is often more dramatic and may make the patient more recepti e to anti-smoking advice However the earlier one begins to smoke the more firmly founded might be the smoking habit and if so this may be of importance in that the IC patients seem to have difficulty in giving up smoking.

In this study we have not had the opportunity to investigate the smoking habits of myocardial infarction patients, but this will be done in further studies

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STUDIES IN ASYMPTOMATIC PRIMARY HYPERLIPIDAEMIA

II *Clinical Findings*

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ABSTRACT A clinical and laboratory examination of abnormalities not attributable to atherosclerosis has been performed on 188 male and 126 female adult subjects with hyperlipidaemia. The sample was re cruited from 20 000 subjects screened at a health control centre who had an initial serum cholesterol and/or triglyceride (TG) concentration above 350 mg/100 ml and 3.50 mmol/l All were subjectively healthy and had no history of atherosclerotic disease Known cases of secondary hyperlipidaemia were ex cluded. Lipoprotein (LP) analysis with preparative ultracentrifugation and electrophoresis was made on all subjects including control groups with "non-elevated" serum lipids. Typing of hyperlipoproteinaemia (HLP) was performed according to the modified system of Fredrickson et al. Compared to controls, subjects who had elevated very low density LP (VLDL) (types II B, III, IV and V) were more obese, while subjects with type II A HLP were shorter There were more smokers among men with type II B and women with type IV HLP than in the control groups. Arcus corneae was seen in 29% of controls and in higher frequencies in types II A and II B. A positiv correlation existed between the frequency of arcus corneae and the mean low density LP cholesterol in the different types. Multiple tendon xanthomata ($n = 11$) were found exclusively in type II A HLP palmar xanthomata ($n = 3$) only in the presence of "floating β-LP" and erupti xanthomata in one male with type V HLP The mean ESR was increased in all types of HLP The Mea S-GPT and uric acid concentrations were higher in type IV HLP in both sexes than in the control groups. In men with type IV HLP S-GPT was positively correlated to VLDL TG. The uric acid level was correlated to both the VLDL TG concentration and body weight independently Of the male subjects with HLP 1/3–1/2 had a diabetic or borderline L glucose toler ance.

Certain clinical features of primary hyper lipoproteinaemia (HLP) are well known One of them is the high frequency of atherosclerotic dis-ease Other well known clinical findings are for example xanthomata There is however no general clinical description of the different kinds of primary HLP The purpose of this paper is to report clinical findings in a sample of apparently healthy subjects who presented with marked HLP at a health control screening. In parallel studies of the same subjects the occurrence of preclinical signs of athero-sclerosis with different locations was assessed (20 1).

METHODS

The sample of subjects with HLP has been described in detail regarding screening procedures, criteria for partici pation and data on lipoprotein (LP) concentrations (19) I brief it consisted of 314 subjectively healthy men and women exhibiting marked hyperlipidaemia i e serum cholesterol above 350 mg/100 ml and triglycerides (TG) above 3.50 mmol/l at routine determination of serum cholesterol and TG, among about 20 000 subjects at a health control centre Reference subjects with non elevated serum lipids were obtained from the same centre Except for the serum lipids the same criteria for participa tion were applied to these subject Around three month after screening, the hyperlipidaemic and control subjec presented at the Lipid Unit, Department of Internal Medicine Karolinska sjukhuset and a second serum lip analyses together with quantitative estimation of cholesterol and TG concentrations in the three LP ... very low (VLDL) low (LDL) and high (HDL) density LP were performed The sera of all subjects were then ... according to Fredrickson et al (10) as modified ... memorandum (1) All determinations were performed ... King Gustaf V Research Institute

For each sex the individuals with HLP were allocated into 6 different groups according to ...

Table I *Age body weight body height weight/height (W/H) index and body surface area (BSA) in men and women in the different HLP groups and in controls (C) (mean±S.E.M and range)*

Group		Age (y)	Body weight (kg)	Body height (cm)	W/H index*	BSA (m²)ᵇ
M						
C	59	48±1 (28–70)	76 1±1 4 (50.5–120.5)	178±1 (16_–197)	0.97± 02 (75–1 44)	1 93± 02 (1.56–_41)
Nᶜ	35	48± (77–64)	77 1±1.8 (52.2–111 0)	177±1 (170–187)	0 99± 02 (63–1.30)	1.94±.02 (1 70–_09)
II A	31	48±2 (3 –68)	73.5±1 9 (58 0–97.5)	175±1 (162 186)	0 98± 02 (76–1.34)	1.88± 02 (1 63–_19)
II B	14	53± (40-64)	77.3±1 (69.0–84 3)	176±1 (164–186)	1 02± 02 (94–1 13)	1 94± 02 (1 78– .08)
III	11	50±3 (32–67)	76.3±2.7 (59.5–88.0)	176±3 (160–188)	1 01± 03 (.81 1 16)	1.92± 03 (1.6_–.08)
IV	94	47±1 (25–70)	82.2±1 0* (63.0–111.0)	176±1 (164–193)	1.09± 01 (.85-1.61)	1 98± 01 (1 73–_37)
V	3	42±6 (36–60)	84.8±8.0 (74 9–100.5)	180±1 (178–181)	1 06± 09 (96–1_4)	.04±.08 (1.97–_20)
Wome						
C	69	5 ±1 (24–73)	61 0±1 0 (46.5–80 0)	165±1 (15 –179)	0.94± 01 (79–1.33)	1 67± 01 (1 47 1 97)
Nᶜ	31	54± (25–66)	6_.9±1 7 (43.3–89 0)	164±1 (157 178)	0 99± 03 (.69–1 44)	1.68±.02 (1 43–1 94)
II A	46	56±1 (3 71)	60.5±1 1 (47 0–82.0)	162±1 (150–173)	0 97± 02 (78–1.32)	1 64± 03 (1 42–1.95)
II B	12	56±2 (48–62)	63.3±_9 (51.2–89.0)	162± (150–170)	1 02± 04 (.88–1 41)	1 67± 04 (1 45–1.94)
III	6	53±3 (43–64)	72.3±4 9 (56 0–91.5)	165±3 (155–177)	1 12±.07 (90–1 43)	1 79±.06 (1.54–1 98)
IV	30	54±1 (42–63)	68.8±1.8 (51.5–92.2)	163±1 (154–175)	1 09±.03 (70–1.38)	1 74± 02 (1.51– 01)
V	1	41	52.7	163	0.84	1.55

Calculated as $\frac{\text{body weight (kg)}}{\text{body height (cm)}-100}$

Calculated as (body weight kg)^0.425 (body height, cm)^0.725 × 0.007184 (4)

Normal type at LP determination despite hyperlipidaemia at screening.

—significant difference against control groups at 5 1 nd 0 1% level respectively

ble 1) In spite of the high cut-off points for the initial (screening) serum cholesterol nd TG values at the health control centre a number of subjects were classified as having a normal LP pattern based pon the results for serum LP concentrations determined on the sample taken at the Lipid Unit I further analysis of the data these subjects will be treated as one group of HLP named N

The interview and examination followed standardized questionna re comprising preceding illness, present diovascular ymptoms, current drug treatment, family ry smoking nd alcohol habits. Persons smoking _ pipe or cigars daily at the time of the screen- for serum lipid were classified a smokers Those ho had stopped moking before that occasion but had smoked for t least fiv years were regarded as xsmok ers All subject were sked about thei monthly rage consumption of spirit and wine These figures were con- erted nto grams of alcohol One bottle (75 cl) of wine and spirit w s considered equal to 60 nd 250 g absolute alcohol respectively

Beside rout ne physical examination special attention was directed tow rds the finding of stigmata of HLP Arcus corneae was diagnosed wheneve distinct white or grey arc or complete circle ould be clearly seen nside the peripheral margin of the ornea Palpebral xanthelasmata were divided into single and if at least two on eyelids multiple The presence of tendon xanthomata was as sessed by inspection and palpation of typical locations and was divided I to solitary and multiple xanthomata.

Body weight was measured w thout coat nd jacket and 1/2 kg was subtracted for the clothes Body height was determined without shoes Based on these measurement weight/height (W/H) inde and body surface area (BSA) were calculated

Except for the ESR determined at the Lipid Unit by the Westergren method in plastic tubes, all non-lipid method reported here were routine methods of the Department of Clinical Chemistry Karolinska sjukhuset. Thus no special attention wa paid to these determinations s being part of research project During the course of the study no major changes of the methods were made and samples from the control subject were interspersed with samples from HLP subjects.

Hb wa analysed as cyanmethaemoglobin. S-GOT and S-GPT were determined enzymatically on a reaction rate analyser LKP 8600 Serum uric acid wa determined by the uricase method I glucose tolerance test (IVGTT) wa performed in the morning after n overnight fast The evening before IVGTT the subject were given 50 g glu- cose by mouth Twenty-five g glucose was give in- travenously The est mation of the half-life ($T_{\frac{1}{2}}$) of glucose w based on 10 blood glucose determinations of blood samples drawn with 5-m intervals beginning 15 min after the injection The el m nation rate k value wa de- termined according to the equat on $k = \frac{0\ 693\ 100}{T_{\frac{1}{2}}}$ (13).

Blood glucose wa determined by the glucose o idase method

Conventional tatistical method were used for the calculations of the rithmetic mean and S E M For com- parison of quantitative data Student t test unpaired da ta, was used To compare the difference of proportion the F sher exact probability test (26) w used To de-

scribe the joint relationship of single variable to one or several other variables regression analysi w applied. In the present paper tests for significant differences between controls and ten HLP groups have been performed for great number of variables. By chance alone 5% of the t-values can be expected to be higher than 1.96 or lower than 1.96 even if there were not true differences of any kind between the HLP groups and controls. Statistical significance at the 5% level should therefore be interpreted with caution.

RESULTS

Anthropom tric data (Table I)

In men no difference in age was found between the control and HLP groups whereas women with type II A were older than control women. On an a erage the females were 3–8 years older than the males. Men and women with type IV HLP were on an average 6 and 8 kg respectively heavier than controls. Mean body height did not differ between these groups and the controls but both men and women with type II A were shorter than the controls. Mea weight/height index in men w th type II B and in both men and women with type IV HLP was higher than in controls indicating that they were more obese Mean BSA was larger in men and women with type IV HLP

Smoking habits (Table II)

Among men there was a higher proportion of smok ers in types of HLP characterized by elevated VLDL TG concentrations (types II B III IV and V taken together $p<0\ 05$) compared to the control group The male controls as well as males in all HLP groups moked about 15 cigarettes daily The mean number of years smoked and the percentage of inhalers did not differ between cont ols and HLP groups About 10% of the men in each group smoked a pipe Male exsmokers were most fre quently seen in the N group

Among women with HLP smoking was most fre quent in type IV The mean consumption among the controls and the women in all HLP groups was 14 cigarettes daily

Arcus corneae

As the occurrence of stigmata of HLP (Table III) did not differ essentially between the sexes the results are given for males and females togethe divided into different types of HLP and controls The only HLP stigma found in the control group

Table II *Smoki g habits in the different HLP groups and in controls (C)*

Group		Smokers (%)	Ex smokers (%)
Men			
C	59	46	7
N	35	43	17
II A	31	48	10
II B	14	86	7
III	11	64	9
IV	94	57	5
V	3	67	0
Women			
C	69	25	6
N	31	39	7
II A	46	30	2
II B	12	33	0
III	6	17	0
IV	30	63 **	10
V	1	100	0

*** = significance at the 5 and 0 1% level against control group.

N see footnote to Table I

Table III *Stigmata of HLP (%) in different HLP groups and in controls (C)*

Group		Arcus corneae	Xanthelasmata		Tendon xanthomata		Palmar xanthomata	Eruptive xanthomata
			All	Multiple	All	Multiple		
C	128	29	0	0	0	0	0	0
N	66	50**	6	0	8	0	0	0
II A	77	69*	10**	3	45 **	14**	1	0
II B	26	54	19**	4	3	0	0	0
III	17	29	6	0	0	0	1	0
IV	1 4	38	2	1		0	0	0
V	4	25	0	0	0	0	0	25

*** = significant differences against the control group 1 5 1 and 0 1% level respectiv ly
N see footnote to Table I

Table IV *Serum lipids lipoproteins and clinical findings in subjects with xanthomata*
Chol = cholesterol (mg/100 ml), TG = triglycerides (mmol/l)

			Screening values			Lipoprotein concentration						
						VLDL				LDL		HDL	
Subj no	Sex	Age (y)	Chol	TG	Type of HLP	Chol	TG	Chol/TG	β	Chol	TG	Chol	TG
Multiple tendon xanthomata													
1	♂	32	453	2.12	II A	15	0 60	.5	–	385	0.80	52	0 37
	♂	34	404	1 64	II A	15	0 45	33	–	263	0 6.	37	0 13
3	♂	35	436	.4	II A	37	1 14	3	–	289	0 71	30	0 15
4	♂	35	411	1 44	II A	.3	0 41	56	–	265	0 74	49	0.21
5	♂	45	501	1.37	II A	18	0.5	35	–	417	1 13	53	0.23
6	♂	52	356	1.83	II A	11	0 43	76	–	237	0 41	60	0.20
7	♂	55	416	1 64	II A	22	0.82	27	–	262	0 73	54	0.24
8	♀	41	468	1 78	II A	23	0 70	33	–	307	0 71	53	0 16
9	♀	56	394	0 99	II A	5	0.20	.5	–	366	0 44	85	0 18
10	♀	61	502	1.99	II A	77	0 93	29	–	398	0.70	62	0 44
11	♀	63	458	1.81	II A	25	0 99	.5	–	276	0 60	58	0.28
Palmar xanthomata													
1	♂	56	357	3.31	II A	53	1 18	45	+	224	0.50	45	0.25
2	♂	57	464	14 10	III	287	10.25	28	+	99	0.29	38	0.26
3	♂	59	351	5.39	III	119	2.73	44	+	133	0 62	35	0 22
Erupth xanthomat													
1	♂	36	385	36 6	V	319	64 9	5	–	49	0 72	2.	0 56

Expressed according to the Minnesota code
Inclination time at digital pulse plethysmography of the lower limbs.
Left bundle branch block.

was arcus corneae present in almost 1/3 of the cases Subjects with type II A had the highest frequency of arcus followed by the type II B and N groups while it was slightly less common in type IV HLP A correlation existed between the frequency corneal arcus for each group and its mean LDL lesterol ($r=0\ 93$ $p<0\ 001$). Complete circular was most often seen in types II A (17%) and ıB HLP (1 %) Xanthelasmata were found in all HLP groups including the N group most frequently in type II B

Tendon xanthomata

Tendon xanthomata were seen mostly but not exclusively in type II Three subjects with tendon xanthomata in the N group and in the type IV group had initial serum cholesterol concentrations above 350 mg/100 ml

Multiple tendon xanthomata (Table IV) were found in 11 subjects exclusively with type II A HLP: they will be called II A X subjects (6). Twenty two per cent of the men and 9% of the women had II A X Mean age of the 7 II A X men was 9 years below that of other males with type II A HLP and 4 of them were below 36 years of age The mean age of II A X women was similar to that of the other type II A women Initial serum cholesterol concentrations were above 400 mg/100 ml in all but two of the II A X subjects In nine II A X subjects initial serum TG was below ? 00 mmol/l In type II A X men mean initial serum cholesterol was around 50 mg/100 ml higher than that of the other subjects with type II A. The corresponding figure for women was around 100 mg/100 ml

For both male and female II A X groups the mean total serum cholesterol concentrations decreased from the first (screening) to the second (LP analysis) determination to the same degree as for the whole II A groups i.e around 10% for the males and 6% for the females The higher total cholesterol concentrations in II A X subjects were attributable to higher LDL cholesterol concentrations in this condition as neither VLDL cholesterol nor HDL cholesterol differed greatly between II A X and the other type II A subjects In both male and female II A X groups initial serum TG was lower than in the remaining type II A population.

Exercise ECG ST* depression	IT* (csec)	ESR (mm/h)
0	9.5	11
4.1	11.5	9
0	10.0	34
0	10.[illegible]	10
0	11 0	1
4 3	10 8	8
4 7	11.0	23
4 2	10.5	15
4 1	10 0	18
4 7	12.5	9
[illegible]	12 0	8
0	10.5	18
4 1	11.8	10
4 4	17.8	48
4.3	9.5	21

This corresponded to a lower VLDL TG concentration in II A X. The cholesterol/TG ratio in VLDL was often high in these subjects (19)

The prevalence of ST segment depressions during exercise ECG (20) and signs of peripheral vascular disease at digital pulse plethysmography (21) were assessed in all HLP subjects. Two of the seven men and two of the four women with II A X had "ischaemic" ST segment depressions during exercise (4 1–4 4 according to the Minnesota code) The oldest type II A-X woman had a left bundle branch block.

None of the II A X individuals had pathological digital pulse plethysmogram in the study of the peripheral circulation indicating absence of signs of peripheral vascular disease

The cause of death of the 22 parents of II A X subjects was known n 12 cases Six of them had died from myocardial infarction, the fathers ($n = 5$) at a mean age of 70 and the only mother at an age of 83 Seven cases of myocardial infarction were known among othe relatives i three of them before the age of 60 Angina pectori was also know in seven cases in five of them before the age of 60

Intermittent claudication among relatives of II A X individuals was known in two cases above the age of 60

Palmar and eruptive xanthomata

Two type III males and one male w th serum typed as II A HLP had palmar xanthomata (Table IV) Both the serum cholesterol and TG of the latter subject were elevated at screening By the time of the LP determination his total TG had however decreased to 28 mmol/l and the VLDL TG concentration was below our arbitrary upper normal limit (1.25 mmol/l). LDL cholesterol was above the upper normal limit (220 mg/100 ml) VLDL cholesterol was 53 mg/100 ml giving a high VLDL cholesterol/TG ratio Electrophoresis howed a 'floating β-LP' This subject thus had a clinical picture of type III HLP but his serum was typed as II A in spite of the presence of a 'floating β-LP This was a consequence of the current criteria for classification of HLP (1) as his VLDL TG were not elevated while LDL cholesterol was Two of the subjects with palmar xanthomata had ST segment depressions during exercise and one had a patholog ical pulse plethysmogram indicating subclinical peripheral vascular disease

One of the type V subjects had eruptive xanthomata of elbows and buttocks His exercise ECG showed ST segment depressions

Laboratory findings (Table V)

Men with type IV and women w th type IIB HLP had higher Hb concentrations than controls. Thus increased Hb concentrations were seen in HLP types characterized by elevated VLDL TG concentrations. No correlation was found between VLDL TG and Hb concentration.

Mean ESR was higher in males with types II A IIB and IV HLP and in females with types N II A IIB III and IV HLP

S-GOT was in general not elevated i HLP I type IV HLP S-GPT was elevated compared with the controls i both men and women (Fig 1) Because of the skewed distribution of S-GPT a significance test was also performed on logarithms of S-GPT The results were equal Th increase was due partly to a shift of the whole distribution of type IV S-GPT towards higher levels and partly to a great r number of markedly elevated S-GPTs in type IV men and women. The de S-GPT on age body weight alcohol

Table V *Laboratory findings in subjects with different types of primary HLP and in controls (C) (means ±S.E.M.)*

Group	*n*	Hb (g/100 ml)	ESR (mm/h)	S-GOT (U/l)	S-GPT (U/l)	Uric acid (mg/100 ml)	IVGTT k %/h	IVGTT %<1 10*
Men								
C	59	14.8±0.1	9 7±1.0	11.5±1 0	9.8±0.9	5 1±0 1	–	–
N	35	15 1±0.2	12.1±1.5	1_7±1.8	10.3±0.8	6.0±0_**	1.33±0 09	31
II A	31	14.5±0 1	17.9±2.6	10.2±1.0	8 9±0.6	5.6±0.3	1.39±0 10	32
II B	14	14.8±0.3	31.3±6.6	10.3±0.9	10.6±1.3	5.2±0.3	1 45±0 19	36
III	11	14.6±0 4	17 4±4.6	9 7±0 4	10.5±1.5	5.0±0.2	1.24±0.13	45
IV	94	15_±0 1	13.3±1.3	11.6±0.5	13 4±0.8	6 0±0.1 **	1.24±0.05	43
V	3	15 7±0.2	33 7±9 1	12.0±1.2	11.3±1.2	5.9±0 4	1.02±0.12	67
Women								
C	69	13.5±0 1	12.0±1 1	8.5±0.3	7.2±0.3	3 7±0 1	–	–
N	31	13.8±0.2	19.3±_5	9 8±0.7	8.6±1 1	4 1±0.2	1 73±0 08	3
II A	46	13 6±0_	22.7±2.1	9.5±0.4	7.3±0.4	3 9±0_	1.51±0.09	20
II B	1	14 1±0.2	28 0±4 7*	8 9±0 7	10.1±1.9	4.3±0 4	1.33±0.16	30
III	6	13.6±0.5	30 0±4 4	11.2±1 7	10.3±1 9	5.3±0 4	1 49_0.19	17
IV	30	13 9±0_	4 9±2.6	11.3±0.8*	11 1±1.0*	4.9±0.2	1 47±0 11	30
V	1	15 0	11	76	18	4.8	2.46	0

= significant differences against C group at 5 1 and 0 1% levels respectively
Percentage of subjects with diabetic or borderline glucose tolerance.

N see footnote to Table I.

TG and serum uric acid concentration was studied by multiple regression analysis. In type IV males S-GPT was significantly negatively correlated to age and positi ely to VLDL TG In females with type IV and male and female control groups no correlations were found

Uric acid was increased in male N group and in th the male and female groups with type IV This s further studied by regression analysis using age body weight VLDL TG and S-GPT as independent ariables In both men and women (controls+all HLP) the serum uric acid concentration was dependent on body weight ($p<0.0?$ and $p<0.005$ respectively) In addition in females it was also dependent on VLDL TG ($p<0.005$) and alcohol intake ($p<0.01$) If the same calculation was applied to type IV males only VLDL TG was significantly correlated with serum uric acid ($p<0.02$)

IVGTT

For practical reasons IVGTT could be performed only in subjects with HLP No significant differences of mean *k* values were found between different HLP groups The proportion of pathologic and borderline *k* values tended to be higher in groups with VLDL TG elevation.

DISCUSSION

In the different HLP groups men were generally 3–8 years younger than women. This was probably in part due to the higher age of the women attending the health control centre The mean age of women was approximately two years above that of men in the basal health control population. In addition selective mechanisms could partly explain these age differences within the HLP sample as it is well known that in general ischaemic heart disease in men precedes that of women by several years (15). All subjects with overt cardiovascular disease were excluded from this study

HLP subjects with VLDL TG elevation were more obese than controls This is in agreement with the clinical fact that subjects with hyper triglyceridaemia are often moderately overweight. In this study both male and female type II A subjects weighed less than controls. This corresponded to a shorter mean body height in this type of HLP

The percentage of smokers in male and female control groups is in agreement with investigations on smoking habits of the populations of corresponding age groups in large Swedish cities (28). The proportion of male smokers in the groups with elevation of VLDL TG (type II B III IV and V) was

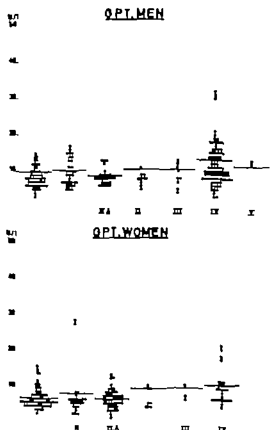


Fig 1 S-GPT I men and women with different types of hyperlipoproteinaemia and in controls (C) with non-elevated serum lipids. *N* = subjects with normal lipoprotein pattern in spite of hyperlipidaemia at screening. The horizontal lines indicate mean values.

higher In this city the serum TG levels for male and female smokers have been shown to be higher than for non-smokers (7) Similarly Gofman et al (11) reported an elevation of VLDL in young men smoking more than 20 cigarettes a day *One possible* mechanism behind this finding is that smoking increases the mobilization of free fatty acids (FFA) from adipose tissue (14) FFA could in turn be extracted by the liver (3) incorporated into liver TG and secreted as VLDL TG (24) Another possibility is that smokers and non-smokers have constitutional differences which also influence the serum TG concentrations

The occurrence of stigmata of HLP in different types has been extensively reviewed before (27) The most prevalent HLP stigma seen in this study was arcus corneae. It was also seen to a great extent in subjects with non-elevated serum lipids It could therefore be questioned whether arcus corneae really should be called a HLP stigma. Its association with serum lipids has also been questioned (9 16 23) However as there was a highly significant correlation between mean LDL cholesterol for each HLP group and for the controls and the frequency of corneal arcus, it is conceivable that the concentration of LDL cholesterol has a direct influence on the deposits in the cornea. It seems therefore justifiable to call arcus corneae a HLP stigma but the 'threshold LDL cholesterol' concentration—if *any—for arcus formation must be much lower than* the arbitrarily chosen cut-off point for type II HLP The clinical importance of arcus has furthermore been emphasized recently (25) by the finding that it *is a risk factor for coronary heart disease* independent of both serum cholesterol and age

Also palpebral xanthelasmata were found at moderate LP elevation of different types but most often in types II A and II B

In contrast to arcus and xanthelasmata, HLP stigmata like tendon xanthomata, palmar xanthomata and eruptive xanthomata were relatively rare and were accompanied by high LP concentrations *Multiple tendon xanthomata were only seen* in type II A HLP

Eleven subjects with type II A HLP had multiple tendon xanthomata (II A X) Studies from Scandinavia have shown that tendinous xanthomata are associated with hypercholesterolaemia and premature ischaemic heart disease (12 17) The males with II A X in the present study were younger than other type II A males The reason for this was probably that older II A X men already had developed symptoms of cardiovascular disease and thus had been excluded from this asymptomatic sample of HLP subjects However the oldest II A X man was 55 and the oldest woman 63 years of age, indicating that marked hypercholesterolaemia could be symptomless up to a relatively old age Signs of coronary artery disease were frequent in this group (20) while signs of peripheral vascular disease were absent (21) Half of the deceased parents of II A X subjects had died from myocardial infarction These findings are probably consistent with a genetic transmission of type II A X

One case with eruptive xanthomata was seen in conjunction with extreme VLDL TG elevation Palmar xanthomata (3 cases) were only seen
presence of "floating β-LP" though one
as II A HLP Based on this material and

ence from other studies (10 _2) HLP stigmata found at a health control might therefore be divided into *general* (arcus corneae xanthelasmata) and *diagnostic* (multiple tendon xanthomata = type II A palmar xanthomata = type III HLP and eruptive xanthomata = (type V or severe type IV HLP)

Men with type IV and women with type IIB HLP had higher Hb concentrations than controls. The differences observed in this study between HLP and control groups are small and inconclusive Increased Hb concentrations were only seen in groups with increased VLDL TG concentrations As Hb is determined spectrophotometrically and VLDL absorbs light high VLDL concentrations might give rise to falsely high readings This might at least in part explain the higher Hb values seen in HLP

The high ESR found in HLP is discussed else where (5) and is not believed to be caused by the LP interfering with ESR but to reflect underlying vascular disease

Both men and women with type IV HLP had higher mean S-GPT values than controls This might reflect a higher extent of damage to the liver *cells in type IV HLP Such damage might reflect* a greater exposure to agent affecting the liver on the one hand and on the other a greater susceptibility of the liver cells to those agents in type IV HLP One possible mechanism for the first alterna ve is an increased alcohol consumption among IV subjects Among men with type IV HLP *was a higher proportion of subjects (51%)* g onsumption of 250 g alcohol or more monthly than among controls (32%) ($p<0.01$) Among w omen with type IV HLP there was an insignificantly higher proportion of subjects stating a consumption of 1.5 g alcohol or more monthly than among c ontrol However no correlation ex*isted either between alcohol intake and VLDL TG* or alcohol intake and S-GPT Therefore no cause effect relationship could be established between alcohol consumption and elevated VLDL TG concentrations or liver damage in the present study

Serum TG levels are reported to be elevated in gout (8). In subjects selected on account of this diagnosis no correlation existed between serum TG and serum uric acid levels Berkowitz ( ) reported higher uric acid levels in patients with hypertriglyceridaemia and a significant correlation between serum TG and uric acid levels in these subjects. Furthermore a positive association between alcohol consumption and elevated serum uric acid level is well known (18)

Although all subjects with gout were excluded in the present study (19) both male and female type IV groups had increased serum uric acid concentrations when compared to the control groups This was further analysed by multiple regression The uric acid level was significantly related to body weight and VLDL TG concentrations, but not to age In females it was also correlated to the stated alcohol intake This suggests that the uric acid concentration independently is determined by several mechanisms including body weight alcohol consumption and VLDL TG in subjects with HLP

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STUDIES IN ASYMPTOMATIC PRIMARY HYPERLIPIDAEMIA

III *Physical Working Capacity*

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ABSTRACT One-hundred-and-sixty male and 123 female subjects with asymptomatic primary hyperlipidaemia (HLP) selected from a health control centre have been studied with a heart rate (HR) controlled exercise test. The serum cholesterol and/or TG at screening were above 350 mg/100 ml and 3.5 mmol/l, respectively As a reference group 49 male and 60 female age-matched subjects from the same centre with serum cholesterol below 300 mg/100 ml and TG below 2.00 mmol/l were investigated using the same technique Quantitative lipoprotein (LP) analyses and typing were performed on all HLP and control subjects. The subjects were divided into two age groups 36–50 and above 50 years of age Men with all types of HLP had lower working capacity expressed in W_{170} or W_{max}, than controls, most pronounced in the younger age group. Younger women with type II A had lower working capacity than their controls. After correction for variation in body weight and age there remained a significantly lower W_{max}, in male types II A (11 %) and IV (21 %) and female type II A (11 %). There was no difference in systolic BP during exercise between controls and HLP The total exercise time and final HR did not differ in control and HLP subjects. Dynamic spirometry was performed in 102 of the male subjects and a significantly lower vital capacity was found in HLP subjects compared with controls after correction for variation in age, weight and height. No differences were found in the maximal flow values. The observed differences in working capacity between controls and HLP subjects are explained by a difference in stroke volume. The lower stroke volumes in male types II A and IV and female type II A could be explained by a lower degree of physical fitness, by a common genetic factor resulting in HLP and decreased stroke volume or by a less effective myocardial function in HLP subjects.

Hyperlipoproteinaemia (HLP) has been shown to be of importance for the development of ischaemic heart disease (IHD) in many clinical and epidemiological studies. The mechanism behind this association is most probably through an increase in coronary atherosclerosis promoted by HLP

There is an association between serum lipids and physical activity Physically active subjects have lower cholesterol and triglyceride (TG) levels than others (6 8 11). Middle-aged runners are reported to have lower serum TG and low density lipoprotein (LDL) cholesterol concentrations but higher high density lipoprotein (HDL) cholesterol concentrations than others (28) Very little is however known about the physical working capacity in subjects with hyperlipidaemia.

In a study of asymptomatic subjects with primary HLP (2?) an exercise ECG was performed to determine the frequency of ST segment depressions during exercise In the present study the physical working capacity was evaluated in these subjects as the design of the exercise test also permitted a good estimation of the functional capacity The results were compared with a reference group with non-elevated serum lipids Quantitative lipoprotein (LP) analysis was performed on all HLP and control subjects in order to relate differences in working capacity to LP concentrations

SUBJECTS AND CHEMICAL METHODS

The sampling procedure and the methods for serum lipid and LP determinations have been reported elsewhere (21) Fasting serum lipids were analyzed on about ?0 000 subjects attending a health control centre Two-hundred-and-eighty-three subjects above 35 years with serum cholesterol levels above 350 mg/100 ml and/or serum TG above 3 5 mmol/l but without diagnosis or symptoms of secondary HLP cardiovascular disease diabetes or

Table I. *Anthropometric data in subjects with diffe ent types of HLP and in controls (C)*

	Men			Women		
Group	*n*	Body weight (kg)	Body height (cm)		Body weight (kg)	Body height (cm)
C	49	74.8±1 4	178±1	60	61.0±0 9	165±1
N	30	76.2±1 7	177±1	30	61 9±1 6	163±1
II A	?6	72.9 ...1	175±1	45	59.8±1 1	16. ±1
II B	14	76 0±1.3	176±1	12	62.5±2 9	16. ±2
III	9	75.3±3..	175±3	6	71 7±4.3	165±3
IV	81	79.8±1.0*	175±1	30	68.0±1.8	163±1

= significant differences against the control groups at the 5 1 and 0 1% levels, respectively
N indicates subject with normal LP type despite hyperlipidaemia at screening.

hypertension were investigated None w on chronic drug treatment. One-hundred-and-nine age-matched male and female control subjects from the same health control centre with serum cholesterol below 300 mg/100 ml and TG below .0 mmol/l were offered the same investigations. Except for the serum lipids the same criteria for participation were applied to them. The number of and reasons for exclusions of HLP and control subjects are given elsewhere (21).

About three months after the hyperlipidaemia had been diagnosed in a subject the fasting concentrations of cholesterol and TG were determined n each of the very low (VLDL) low (LDL) nd high (HDL) density LP class after separation by preparative ultracentrifugation (7). LP paper electrophores was run on whole serum and on top and bottom fractions after separation t d=1.006 Typing of HLP wa performed according t Fredrickson et al (1) a modified by Beaumont et al ( )

All HLP and control groups re divided into tw age groups 36–50 (younger group) and above 50 years (older In none of the HLP groups did mean age differ ficantly from that in the control group. I pite of the *cut-off point for the initial (screening) serum* lesterol and TG alues at the health control centre umber of ubject were classif ed as having normal LP pattern based upon the result for serum LP concentrations determined on the sample aken at the Lipid Unit The possible reasons for this ha e been discussed (?1). In the further analysis of data these subjects w ll be treated as a separat HLP group named N

All subjects were interviewed according to a standardized questionnaire including subjecti e estimate physical activity off work. If they tated that they had had regular physical training sufficient to cause breathlessness at least once a week for the last year they were regarded as physically active subject

PHYSIOLOGICAL METHODS

Exercise tests were performed in the sitting position on an electrically braked bicycle ergometer (EM 3?0 Siemens-Elema, Sweden) controlled by a heart rate (HR controller unit (EMT .52, Siemens-Elema) which automatically adjusted the work load of the bicycle so that ertai pre selected HR during work could be obtained (9 10) By this method direct measure instead of an intra- or extrapolated was achieved for the work performed at a HR of 130 (W_{130}), 150 (W_{150}) and 170 (W_{170}) beats/min. W_{150} was used an expression for working capacity The subjects exercised for 6 min on consecutive loads beginning with a HR of 90 beats/min in individuals above 56 years of age and with 110 beats/ml in younger subjects, increasing every 6 min by 20 beats/min The exercise w s continued until fatigue or in few cases earlier if marked ECG changes were observed The work load and HR were continuously recorded on an ink-pen recorder (Servogor) and the total work performed was calculated by electrical integration. The ECG recordings comprising 6 limb leads and 6 chest leads (CR) were taken before and after exercise. During exercise 6 chest-head leads (CH) were recorded continuously The ECG was recorded on an ink jet recorder (Mingograf 61) with a time constant of 3 sec and fre quency response up to 700 Hz. The same ergometer w s sed during the whole study and the calibration of the ergometer wa regularly checked The performance of the ECG recorder was also checked several times with re*spect to frequency response and accuracy of paper speed* The diagnostic ECG was recorded at a paper speed of 50 mm/sec. The ECG at rest and during ercise wa interpreted by one of us (L.-G E.) without knowledge of whether the ECG was recorded from a control or hyperlipidaemic subject. A magnifier (5 times) was used for the visual interpretation of the ECG recorded at paper speed of 50 mm/sec and the ECG changes were coded according to the Mi nesota criteria adopted for CR leads (23). BP was measured in the left arm with the cuff method using calibrated manometers.

Before exercise resting ECG HR and the systolic and diastolic BPs were noted with the patient in the supine position after 10 min rest. During exercise systolic BPs were measured during the 5 mi at each HR level. Breathing frequency was counted during the 3 min Between resting ECG and exercise standardized orthostatic test wa performed with 8 min standing and recording of HR, ECG and BPs after 8 min.

Ventilatory test were performed in 7? male HLP and 30 male control subjects with spirometer as described by Bernstein et al (4) modified to give an electrical signal output. Th signal was analyzed by a comput r program

on an IBM 1800 and values for static vital capacity (VC) including forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV 1) and maximal voluntary ventilation were obtained together with corresponding normal values derived from age, sex, height and weight (3 14)

A chest X-ray with determination of relative heart volume (in standing position) was performed within the clinical routine of the hospital.

Statistical methods according to Snedecor and Cochran (27), Siegel (24) and Hald (15) were used and the data were processed on computer model Hewlett-Packard 2000E. In the present paper tests for significant differences between controls and several HLP groups divided into three age categories have been performed for number of variables. By chance alone 5% of the *t* values can be expected to be higher than 1.96 or lower than 1.96, even if there were no true differences of any kind between groups of HLP and controls. Statistical significance at the 5% level should therefore be interpreted with caution.

RESULTS

Anthropometric data The relevant anthropometric data are given in Table I The results are discussed elsewhere (20)

Physical activity (Table II) No significant difference in stated regular physical activity during time off work was noted in males. Control women stated regular physical activity significantly more often than any HLP group except type III

Circulatory data

At rest Mean resting HR and systolic and diastolic BP in the different groups are given in Table III Men with type IV HLP in the younger group had higher resting HR than controls. In this group of HLP there was an inverse correlation between resting HR and working capacity (W_{130}) and the higher resting HR in type IV men might be fully related to their lower working capacity The same group of HLP subjects also had a higher systolic BP than controls No correlation between resting HR and systolic BP within this particular HLP group was found However a correlation was found between mean resting HR and mean resting systolic BP in the different HLP groups of 36–50-year-old men (r=0 71) (Fig 1) indicating that the increase in BP was parallel to the increase in HR. Women with type IIB and aged above 50 years had higher systolic and diastolic BPs than the controls which was independent of HR

Orthostatic test The mean standing HR and systolic BP and the difference in HR and systolic BP between standing and supine positions are given in Table IV Men with type IV HLP had a higher standing HR than the controls thus being related to the higher HR at rest. The orthostatic reaction evaluated by the change in HR and systolic BP from supine to standing did not differ between the different HLP groups compared with the control groups

The prevalence of ST T changes during the orthostatic test (22) was higher among males with type IV HLP than among controls This concerned mainly the smallest changes in T (code 5 4) which was consistent with the higher HR in this group (25).

Exercise test The work loads at different HR in controls and HLP subjects are given Table V Men with all types of HLP had lower mean working capacity than controls This difference in working capacity was most pronounced in the younger age groups Women with type II A had lower working capacity than the controls.

Total exercise time final load (W_{end}) and final HR (HR_{end}) are given in Table VI The total exercise time did not differ significantly between controls and the different HLP groups W_{end} was significantly lower than in the controls in all men with HLP except type III and in women with types N II A and II B HLP HR_{end} did not differ essentially between the control and HLP groups of either men or women

As W_{130}, W_{150} and W_{170} were very closely related to each other and therefore only W_{150} will be commented on below The values for W_{150} were directly obtained in 96% of all subjects In the remaining 4% extrapolated values were used

Using multiple stepwise regression and partial correlation there was a significant correlation between W_{150} and body weight together with age both in men and women. No significant correlation was seen between W_{150} and heart volume If corrections

Table II *Percentage of subjects stating regular physical activity*

Group	Men	Women
C	41	32
N	50	10*
II A	31	13
II B	14	0*
III	22	0
IV	30	6

Symbols as in Table I

Table III. *Resting HR (beats/min) and systolic and diastolic BP (mmHg) in men and women divided into age groups and types of HLP and in controls (C)*

Men

Group	Age (y)	Resting HR			Systolic BP			Diastolic BP		
		All	36–50	>50	All	36–50	>50	All	36–50	>50
C		68±1	67±2	68±2	129±	125±2	134±4	82±1	82±2	82±2
n		49	26	23						
N		70±2	71±3	70_3	133±3	128±4	138±3	80±2	80±3	80±
		30	14	16						
II A		71±	70±3	71±3	129±3	127±4	130±5	82±2	80±3	83±
		26	11	15						
II B		73±3	78±7	68±3	135±4	128±4	141±5	81±_	78±3	83±3
n		14	6	8						
III		70±3	69±6	71±4	133±	127±3	136±	86±2	83±4	88_3
n		9	3	6						
IV		76±	77_	74±3	136±	135±	137±3	85±1	86±1	83±2
n		81	47	34						

Symbols in Table I

were made for the correlation of W_{130} to body weight and age eliminating the influence of those factors there was a significantly lower working capacity in males of types II A and IV 109 and ?13 kpm/min (11%) (1%) respectively than in controls. Also males of type II B and III had numerically lower W_{130} 131 and 131 kpm/min. Among the females only type II A had significantly lower W_{130} 66 kpm/min (11 %) after the same corrections for body weight and age.

The systolic BP during exercise did not differ significantly between male or female controls and HLP subjects although type III had numerically lower values during exercise (Fig. 1).

The respiratory rate at a HR of 130 beats/min was significantly higher in the older male group with type II B (_8 breaths/min) compared to controls (4 breaths/min) in spite of 17% lower work load.

Ventilatory data, mal... Vital capacity (VC) was 89.5% of the expected value for all HLP subjects compared to 97.8% for the control subjects a significant difference ($p<0.01$). The difference was significant for types II A, II B and IV and was most pronounced in type II B. VC 85.1% of expected value ($p<0.01$). A significant difference was found in VC between smokers and non-smokers in type IV ($p<0.01$) but not in the other types or the control group. Forced expiratory volume in one sec (FEV_1) or FEV% did not differ between any of the HLP groups and the controls.

DISCUSSION

In our study we have regularly calibrated the ergometer used and standardized with regard to the relative work intensity and duration of exercise using the HR-controlled equipment. As HR is an expression of the relative work intensity provided that the maximal HR is the same (1), we thus have provided approximately the same relative intensity for all age groups as the older subjects worked at a lower absolute HR.

The oxygen transport capacity or physical working capacity is determined by three hemodynamic factors: stroke volume, arteriovenous oxygen dif

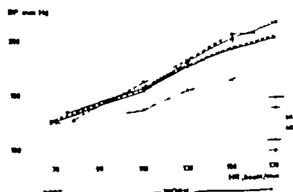


Fig. 1. Mean systolic BP in relation to HR at rest and during exercise in 36–50-year-old men with different types of HLP and in controls.

...ng HR			Systolic BP			Diastolic BP		
...	36–50	>50	All	36–50	>50	All	36–50	>50
...±1	75±2 1	73±2 39	127±2	120±4	130±3	78±1	74±2	80±1
±2	73±5 11	75± 19	131±3	128±6	133±4	81±1	83±3	79±2
...	81±4 8	74± 37	133±3	126±7	135±4	80±1	83±4	80±1
...4	78±4 4	77±5 8	143±7	126±15	152±6**	84±2*	78±3	87±2*
±6	65±8 3	76±8 3	128±11	110±6	147±16	78±4	72±4	83±6
±2	73±4 9	77±2 21	128±3	123±4	130±3	79±	75±5	81±2

ference and HR (1 5 17 26). In the present study e have determined working capacity as the work load which gives a HR of 150 beats/min W_{150}. As HR is kept constant, the variation in W_{150} is thus induced by a variation in either stroke volume or arteriovenous oxygen difference or in both. The size of the stroke volume is mainly dependent on the body dimensions (body weight, heart olume blood volume) and the degree of physical fitness provided that the myocardial function is normal and not depressed by any disease (5 6). Arteriovenous oxygen difference at a certain HR is rather constant for different individuals but lower than normal values are found in persons with high activity of the sympathetic nervous system (16) or to a lesser degree in untrained individuals. W_{150} has been found to be a good indirect measure of stroke volume with a reservation for a change in arteriovenous oxygen difference and provided that the exercise test is carefully standardized (17).

Table IV *HR (beats/min) and systolic BP (mmHg) in standing position and the differenc between standing and supine positions (ΔHR and Δsyst.BP)*

		HR standing			ΔHR standing–supine			Syst. BP standing			Δsyst. BP standing–supine		
Group	Age (y)	All	36–50	>50	All	36–50	>50	All	36–50	>50	All	36–50	>50
Men													
C		81±2	81±2	81±2	14±1	14±	13±2	131±3	128±4	134±4	2±	3±4	1±2
N		84±2	84±3	83±3	13±2	13±3	13±2	130±3	125±4	134±4	−2±2	0±	−3±3
II A		83±3	82±3	85±4	13±1	12±2	13±2	131±4	135±4	127±6	2±2	8±4	−3±
II B		87±4	92±7	84±4	15±1	13±1	16±2	133±4	123±5	139±6	− ±2	3±4	−1±4
III		90±3	89±6	90±4	20±2	20±1	19±3	131±4	127±6	133±4	− ±4	0±3	−3±6
IV		88±2**	90±2*	86±3	12±1	12±1	12±1	135±2	131±	140±3	−1±1	−4±1	3±2
Women													
C		89±2	90±3	89±2	15±1	15±2	16±1	177±3	116±3	133±3	0±2	−4±4	2±2
N		87±3	87±5	87±3	13±	14±1	13±3	129±2	130±5	129±3	−2±2	2±3	−4±2
II A		87±	88±4	87±2	11±1	7±3	12±2	131±3	128±6	132±4	− ±2	2±5	−3±2
II B		90±4	92±5	89±5	13±2	14±3	12±3	138±5	123±12	144±4	−6±3	−1±6	−8±4
III		88±7	86±12	89±10	16±	20±	13±	128±10	118±13	135±14	−6±5	3±8	−1 ±4
IV		90±2	88±4	90±3	14±1	15±3	13±1	126±4	119±5	129±4	− ±2	−3±2	−2±3

Symbols as in Table I

Table V *Physical working capacity expressed as ork load (kpm/min) at a HR of 130 beats/min (W_{130}) 150 beats/min (W_{150}) and 170 beats/min (W_{170})*

		Men								
		W_{130}			W_{150}			W_{170}		
Group	Age (y)	All	36–50	>50	All	36–50	>50	All	36–50	>50
C		815±35 49	859±53 26	766±4 ,3	1 034±44 48	1 079±66 26	981±56 22	1 263±60 40	1 321±85 24	1 176±76 16
N		706±30 29	704±40 14	708±45 15	918±35 29	893±49 14	94 ±51 15	1 174±43 25	1 099±59 13	1 256±56 12
II A		690±33 26	691±4 11	688±49 15	850±39 ,5	864±64 11	840±51 14	1 060±54 22	1 103±76 11	1 018 78 11
II B		636±42 14	610±70 6	655±53 8	831±48 14	830±49 6	832±78 8	1 051±63 11	1 044±42 6	1 059±138 5
III		652±60 9	685±118 3	636_45 6	828±63 9	911±145 3	787±66 6	1 038±137 6	1 221±230 3	856±90 3
IV		656±19 81	673±,5 47	633±29 34	861± 1 80	885±27 47	826±31 33	1 092±27 69	1 107±33 45	1 065±48 24

Symbols as in Table I

A difference in working capacity might be a result of a difference in body dimensions However using stepwise multiple regre sion and partial correlation the difference remained n male types II A and IV and female type II A HLP even when the influence of body weight and ge i compensated for

In the orthostat c st there were no indications of differences n pathetic tone between HLP and control gr up n rms of changes in HR BP or ECG reaction I there should be only minor ference n r enous oxygen difference be een th μ r ng e ercise (16) A difference in stroke volume is therefore a probable explanation of the lower W_{130} value in male types II A and IV and female type II A HLP

Therefore two main possibilities appear as an explanation for the lower W_{130} in these groups of HLP The first is a lower degree of training There were no significant differences in physical activity however between male control and HLP subjects as stated by the subjects in the interview but such data are of somewhat limited value as the same training stimulus can give different effects in different individuals

Table VI *l e lated xercise time (min) highest work load (W_{max}, kpm/min) and the highest HR achiev (HR F /min)*

		M								
		Total exercise time			W_{max}			HR_{max}		
Group	Age (y)	All	36–50	>50	All	36–50	>50	All	36–50	>50
C			22 1	–±1	1 204±51	1 348±71	1 047±59	162±	168±2	154 1
N			22 1	1+1	1 060±46	1 116±62	1 012±66	158±4	167 3	150 5
II A		--	3 1	1±1	957±52	1 125±85	834±44	158±3	170±0	149±5
II B		19	19 1	19	844±60*	953±71	763±83	151±4	160_4	145 5
III		21	4_0	20±1	925±119	1 179±252	798±109	157±6	170±0	150 7
IV		1_	20±1	1±1	1 006±28	1 088±38	842±36	159_	164_2	157 5

Symbols a in Table I

...m			W_{130}			W_{170}		
l	36–50	>50	All	36–50	>50	All	36–50	>50
5±19 9	465±33 20	435±43 39	589±22 57	639±37 20	563±27 37	766±28 48	859±4? 20	700_3 28
7±22	410±38 11	437±27 18	608±33 29	585±47 11	622±46 18	740±38 23	735±82 9	744±38 14
_29	361±41 8	419±34 37	508±21 37	502±27 7	509±26 30	680±24 32	650±_5*** 6	688±29 26
±43 2	388±74 4	395±56 8	574±48 12	545±82 4	589±63 8	714±80 9	636±100 3	752±112 6
2±49 6	458±94 3	405±51 3	568±39 6	589+75 3	547±40 3	69?±35 5	715±59 3	658+8 2
3±21 8	448±29 9	471±28 19	622±21 26	599±30 9	635±29 17	762±35 18	755±47 7	766±51 11

Another explanation for the smaller stroke volume might be a less effective myocardial pump function in HLP. The higher respiratory rate in the older type II B males supports this explanation as an increase in ventilation might be an indication of depressed left ventricular function (13), especially if one considers that this higher respiratory rate was measured at 17% lower oxygen intake (work intensity). The latter explanation is supported by the finding of a lower vital capacity in the HLP groups. A decrease in vital capacity is an early sign of latent myocardial failure (18) and should not be a result of any difference in physical acti ity (1). The latter explanation is further supported by the lack of correlation normally found between W_{130} and heart volume (19, 26). This might be due to the fact that W_{130} was decreased disproportionally in some cases depending on a diminished left ventricular function but the lesser accuracy in heart vol me measurement in standing by the hospital routine might contribute to the lack of correlation. A third factor may be a common genetic mechanism responsible for both the hyperlipoproteinaemia and changes in physiological variables, e.g. W_{130} and vital capacity.

otal exercise time			W_{max}			HR_{max}		
ll	36–50	>50	All	36–50	>50	All	36–50	>50
?±1	22±1	22±1	719±31	864±50	645±35	157±3	168±1	151±4
0±1	19±2	21±1	579±33**	554±43 **	594±47	151±3	152±5**	150±4
0±1	20±2	20±1	563±28***	529±85**	570±29	150±3	158±8	149±3
1	20±3	22±1	623±44	535±44**	667±58	153±3	150±8	155±3
1	20±2	20±2	575±47	631±73	518±51	150±5	157±7	143±7
9±1	20±2	19±1	629±33	706±82	596±31	149±3	161±5	144±5

To really determine which of these explanations is valid a heart catheterization with extensive analysis of flow and pressure measurements must be performed. This has however not been possible in our subjects without clinical symptoms.

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ANTIHYPERTENSIVE EFFECT OF β-1 RECEPTOR BLOCKADE AND β-2 RECEPTOR STIMULATION IN ESSENTIAL HYPERTENSION

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Abstract The antihypertensi e effect of practolol β-1-blocker *alone and i combination with β- -stimulating* drug, salbutamol has been studied in double-blind cross-over trial on 19 patients. Practolol treatment induced significant BP reduction while the addition of salbutamol failed t giv any further decrease

Animal studies have indicated that β-receptors can be divided into two groups Stimulation of the β-1-receptors causes a increase in heart rate (HR) and cardiac output ($\dot{Q}$) and l polysis while stimulation *of β- -receptors gives bronchial smooth muscle* relaxation and peripheral vasodilatation (8 9)

Practolol a β-1-receptor blocker has been shown to have the same BP-lowering effect a propranolol (6) Salbutamol a β- -receptor stimu lant, has a vasodilating effect and its duration on oral administration is –3 times longer than that of isoprenaline (5 4) Theoretically a therapy which combines these two agents would be expected to have a greater antihypertensive effect than prac tolol *alone* because peripheral resistance would be reduced by the vasodilating effect of salbutamol The reflex baro-receptor mediated increase n HR and $\dot{Q}$ would be blocked by practolol

The aim of the present tudy was to determine whether the combined admini tration of practolol and salbutamol ga a better BP reduction than practolol alone

MATERIAL AND METHODS

T enty previously untreated hypertensiv patients with an average age of 51 years (range 40–56), 18 men and women, entered the trial All patient ere re ruited from an ongoing population study (1) and had casual BP higher than 175 and/or 115 mmHg on tw consecutive readings. They were then followed ntil the BP stabilized after 3–6 out-patient controls All had benign essential hypertension The female patients (n) did not use oral contraceptives. One patient was excluded be cause of tablet failure i.e. intak of less than 90% of the prescribed number

All patients were examined and followed at the Hypertension Clinic, Sahlgren Hospital The examination included ECG and X-ray of the chest, urinalysis of albumin and glucose, serum creatinine uric acid and serum electrolytes Eye-ground xamination w done by the investigators BP wa measured in the right arm the supine and standing position after 5 min rest with mercury manometer (to the nearest mmHg) Diastolic BP=phase 5 was used HR was determined by pulse palpation. With this xamination 11 subjects were classified as WHO stage 1 while 8 showed signs of left entricular hypertrophy or eye-ground changes as in WHO stage II

Design of th tudy

All ubjects were treated during run-in period with practolol, 200 mg three times daily for one month There after salbutamol 4 mg three times daily or placebo was added for one month each in double-blind cross-over trial BP and HR were measured at the end of each period

RESULTS

Table I shows the mean BP and HR for the whole group before and after treatment during one month On practolol ystolic and diastolic BP as well as HR were significantly lower than before treatment ($p<0.05$) Table I also shows the average BP and HR after addition of salbutamol and placebo No further BP reduction was achieved with the addition of salbutamol N rthostatic hypotensive reactions or side-effect e g. bradycardia, gastrointestinal symptoms or tremor were oted

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Table I Mean blood pressure and heart rate in 19 patients bef we treatment and during the three treatment periods

	Before treatment			On practolol			On practolol and placebo			On practolol and salbutamol		
	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	HR
I	178 16 7	106 7 6	79 13	163 18 3	101 9 9	67 9 7	159 18.4	98 10 0	67 7 3	160 19 9	97 1	7' 11 1

DISCUSSION

The results from the present study are not encouraging for the use of this drug combination in the general treatment of hypertension It is not clear why no further BP reduction was achieved by the addition of salbutamol to the previous practolol treatment The present oral doses of salbutamol ha e been shown to be adequate to give β- -receptor stimulation in the bronchial smooth muscles Larger doses of salbutamol also carry a greater risk of tremor (3 7)

Possible explanation of the lack of BP reduction on salbutamol might be structural or functional dif -es between the β- receptors in the bronchial and in the peripheral arterioli The data do not whether Q increased when salbutamol was added but the slight (although not significant) increase in HR may ndicate that this was the case

Treatment with β-1-receptor blockers and β- -receptor stimulant seems to be a useful combination in hypertensi e patients with obstructi e lung disease ( ) Further tudies are needed to determine whether larger doses of salbutamol would give BP reduct on in combination with β-1-receptor blockade

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A POSTMYOCARDIAL INFARCTION CLINIC IN GÖTEBORG SWEDEN

A Follow-up of MI Patients in a Specialized Out-patie t Clinic

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Abstract. The registration of all myocardial infarction (MI) in the city of Göteborg started on Jan. 1st 1968 when special clinic was set up for ambulatory posthospital care of infarction patients In 1970 this clinic was expanded to cover all patients below 67 years of age with MI in the city of Goteborg the aim being to standardize and unify patient care and therapeutic regimens, to provide opportunities for the study of patient characteristics natural history risk factors and effects of preventive measures. Results from such studies have been published, but so far no unified description of this special out-patient unit, nor of any similar unit elsewhere Patient recruitment, considerations concerning personnel, patient education procedures and return visit routines are covered, together with investigative methods and criteria for the treatment of complications, symptoms and risk factors. The cumulative drop-out rate up to and including 2 years follow-up wa only 3% A brief bibliography of studies originating at the Postmyocardial Infarction Clinic is included

Patients who survive myocardial infarction (MI) run a great risk of reinfarction. Previous MI may be said to be the dominant risk factor for recurrence (25 30 31 33). There is a great risk of sudden death (29) and in many survivors there is a considerable degree of invalidity (8 3., 38 39). It would therefore be natural to subject postinfarction patients to systematic follow-up and thorough care This has been done but only within selected groups of patients and for certain specific purposes such as the study of the prognoses or secondary prevention by means of for example anticoagulants or die (1 6 14 18 22)

A retrospective investigation of men below 50 years of age who survived their first MI which had occurred between 1948 and 1965 n Goteborg showed that their medical care w th respect to known risk factors such as high BP smoking and hypercholesterolemia, had been very heterogeneous One-third of the patients were not under medical supervision at all (13).

In Göteborg there is only one hospital for the acute care of MI patients which is one reason why there are good possibilities of registering all cases of acute myocardial infarction (AMI) for the purpose of *epidemiological studies (9 10 27) Against this* background it seemed logical to start an ambulatory clinic for uniform follow-up of postinfarction patients—the Postmyocardial Infarction Clinic (9 26 27 34) The establishment of a register for all cases of infarction enabled 90% of all diagnosed surviving cases to be registered though with certain age limitations owing to practical problems of capacity (9 10).

The purpose of the Postmyocardial Infarction Clinic was to enabl in a representative infarction material 1) regular standardized *ambulatory control and treatment* after acute care 2) comparison of data from patients who have suffered MI with data from the total population in order t *characterize the infarctio patie ts* 3) *study of the ourse after MI* 4) identification of factors of importance for the course—*secondary risk factors*

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development of secondary preventive measures (prevention of e g invalidity reinfarction and death)

PATIENTS

The patients were identified while undergoing acute care through the previously mentioned register of all infarction patients. During 1968–69 men born in 1913 or later who had survived an AMI after Jan 1st 1968 were cared for at the Postmyocardial Infarction Clinic. During 1970 and 1971 men from the population of Göteborg born in 1903 or later were cared for with the exception of those born between 1903 and 191 on the 3rd 6th, 9th, 13th 16th 19th, 23rd 26th or 29th of the month (e g a random 30% sample) who were excluded in order to form a reference group The latter patients were given the previous conventional treatment At present patients born in 1914 or later are cared for

STAFF OF THE CLINIC

The medical duties have been shared by six physicians, all of whom serve at the Clinic on a part-time basis. The total medical service has gradually increased and is estimated to 60 h/week for 1974 The nursing service has increased continuously and amounted in 1974 to 40 h/per week for a state-registered nurse and 80 h/week for two assistant nurses. Secretarial duties amounted in the same year to 40 h/week.

PATIENT INFORMATION

During 1968–71 the patients were contacted while receiving acute care by one of the physicians from the Postmyocardial Infarction Clinic The first control examination at the Clinic was held 1–3 weeks after discharge from hospital

When first contacted by the physician from the Clinic the patient was given information concerning his disease and the necessity of regular check ups after discharge The Postmyocardial Infarction Clinic and its activities were described and the patient was offered regular follow-up and control The information included an explanation of what is meant by arteriosclerosis in general and particularly MI The risk factor concept was also discussed In connection with the discussion concerning risk factors, smokers were encouraged to stop smoking The connection between saturated fats and serum cholesterol was explained and the patients were encouraged to reduce their intake of saturated fats Individual instructions concerning the suitable level of activity during convalescence were given to each patient In general a gradual increase in activity in the form of walks was advised commencing with 5–10 min at a gentle pace and avoiding hills and steps. It was suggested that the number and duration of walks be increased from day to day eventually including hills and broken ground The patients were advised to refrain from sexual activity during the first four weeks After an individually adjusted period they were informed that all activity was permitted and indeed desirable as long as it did not cause chest pain, pronounced dyspnea or tiredness during or shortly after the activity in question.

At the first appointment the principles for determining when and whether return to work was possible were explained The patient s occupation and age and the extent of the infarction including complications were taken into consideration in this respect Subsequent discussions concerning return to work were based on these data as well as on symptomatology and the extent of invalidity

In addition to oral information the patients were given printed information in the form of special brochures developed at the Postmyocardial Infarction Clinic Three publications dealing with MI (36) diet (37) and smoking (35) have been issued In addition information was given partly by means of audiovisual aids to groups of 10–15 patients together with members of their families This group information took place 3–4 weeks after discharge As from 1972 the first contact between the patients and the Postmyocardial Infarction Clinic is established prior to discharge from hospital by a nurse giving information and delivering the printed information material

ROUTINES FOR CHECK UP VISITS

Check-up examinations by the physicians at the Postmyocardial Infarction Clinic took place at intervals indicated by the clinical situation. In addition control examinations were always performed at fixed times according to a predetermined schedule During 1968–71 these control examinations were performed for patients born in 1913 or later at 3 12 4 and 60 months after the infarction. During 1970–71 control examinations were performed for patients born between 1903 and 1912 after 1 and 3 weeks and after 3 6 1_ 18 and 4 months and annually thereafter For this group of patients check-up examinations by nurses were tested during these years as a complement to

check-ups by physicians. A specially trained nurse examined the patient every 6 weeks up to and including 24 months after the infarction. She took a standardized case history concerning the period since the previous examination with the emphasis on chest pain decompensation and arrhythmias. She also recorded the BP pulse and weight and performed laboratory tests for Hb ESR serum potassium and glucosuria or proteinuria. In the event of any of the following she contacted the physician responsible for the patient increased angina pectoris, suspected reinfarction and/or decompensation, diastolic BP ≥105 mmHg change of weight, or newly developed irregularity of pulse. The physician was also contacted if serum potassium was ≤3 6 mEq/l and in the event of anemia ESR rise or glucosuria. In addition, the responsible physician was contacted in any situation in which the nurse was in doubt in any way

The organization experience and certain results for 1968–71 have partly been reported previously (4 8, 9 13 26 28 30 34 38) In the light of experience, the present routine for check-ups was developed with fixed controls by physicians after 4 16, 30 and 52 weeks and after 2 and 5 years, and controls by nurses after 8 20 and 40 weeks

INVESTIGATIONS

Blood pressure BP was determined, in accordance with WHO s recommendations (22) at each check up visit in the right arm using a mercury manometer recording to within 2 mm supine and standing min after rising Primarily the pressure was rapidly pumped up to 200 mmHg. Systolic BP was recorded when the Korotkow sounds occurred, diastolic when the Korotkow sounds disappeared (phase 5)

Heart rate From the ECG tracing (see below) 5 R-R intervals were determined The value obtained was converted nomographically to HR. In the event of very irregular rhythm e g. atrial fibrillation, the number of beats/30 cm was counted HR was recorded at each control visit.

Electrocardiogram Leads I II III and AVR, AVL, AVF and CR CR CR_4, CR_6 and CR were registered routinely at each control visit after 5 min rest. The apparatus used was an Elema Schönander type Mingograf 34 paper speed 50 mm/sec. Exercise ECGs on bicycle ergometer with stepwise increases of the load were taken t the 3 12 and 24-month controls for patients born in 19 (24).

Heart–lung X-ray X-ray of the he was performed routinely in the standi g and 1 months after infarction h had suffered infarction between 1968 tients suffering infarction in 197 months after the infarction. Patie farctions in 1977 or later were not X r after discharge since experience ha from a clinical point of view there was perform this investigation routinel T relative cardiac volumes were calculat to Jonsell (15)

Lipids and lipoprotein electropho cholesterol and serum triglyceride termined and typing of lipoprotein ele was performed at 3 1 and 4 months infarction. As from March 1970 these were also performed 6 months after the act farction. The blood samples were taken e morning after 12 hours fasting Serum choleste was determined according to Cramer and Isaksson (7) and serum triglycerides according to Carl n (Serum lipoprotein electrophores s was per med on filter paper mainly according to the thod described by Lees and Hatch (17) and on ag se gel mainly according to Rapp and Kahlke (21) The serum lipoprotein patterns were typed biochemi cally according to Fredrickson and Lees (11) and Beaumont et al (2) A detailed description of the lipid and lipoprotein determinations has been presented previously (12).

Routine laboratory tests At each control Hb and ESR were recorded and a urine sample was taken for albuminuria (Albustix®) and glycosuria (Clinistix®) The patients were also weighed As from 1972, serum potassium was determined at each check-up.

Special investigations Coagulation factors and fibrinolysis were studied in connection with the 3-month and 12-month controls in the groups of patients who suffered infarction i 1968 and 1969 The methods have been described in detail previously (16) Body composition was determined using K-isotope technique 3 and 12 months after the infarction in patients who suffered infarction in 1968 (19) Serum electrophoresis, fibrinogen ANF liver status, serum creatinine and serum potassium were checked at the 6 and 12-month controls in patients born between 1903 and 1912 in the 1970 material

Dietary habits were determined by interview by the same dietician at 3 1? and 4 months after the infarction in the 1968–69 groups

COMPUTER REGISTRATION

For uniform and complete registration of data, special case history forms were prepared for direct transfer of information to punch cards for computer processing (9) These computer case histories comprised personal statistics family history and social circumstances information on certain previous diseases cardiovascular and pulmonary symptomatology data concerning smoking habits and physical activity at work and during leisure hours and information concerning subjective stress Conditions at the time of infarction during the period of acute care and at discharge as well as during follow-up were also registered Details of the case history were obtained with the same questionnaire as was used in the population studies of men and women in Göteborg and which is partly based on international questionnaires recommended by the WHO (?)

TREATMENT PRINCIPLES

The Postmyocardial Infarction Clinic treated pa exclusively on an ambulatory basis which that medication at discharge was not influenced The Clinic subsequently applied fixed criteria for the treatment of symptoms, complications and risk factors *The treatment principles* were continually discussed within the group so that the care of the patients was uniform both as regards criteria for indications and therapy

Angina pectoris Effort-induced pain which was clinically assessed to be caused by myocardial ischemia was treated with sublingual nitroglycerine Patients with severe and/or frequent attacks were treated with β-blockers as well

Decompensation Patients with dyspnoea on exertion increase of weight, oedema of the legs and/or venous stasis with or without X-ray signs of heart failure were given digitalis in the first place In cases in whom this was considered to be insufficient saluretic diuretics and potassium supplements were also given and if this was inadequate aldosterone antagonists or a similar drug was also given

Arrhythmia In the event of arrhythmia the adequacy of treatment for decompensation the presence or absence of hypokalemia and the possibility of the arrhythmia being drug-induced were considered Conventional antiarrhythmic treatment was given Ventricular extrasystoles were not treated unless special indications were present (e g paired ectopic beats during effort).

Hypertension Patients below 55 years with a systolic BP ≥160 mmHg and/or diastolic BP ≥105 mmHg and patients above 55 years with a diastolic BP ≥105 mmHg on two subsequent occasions were given antihypertensive treatment When pressures at these levels were first recorded the patient was booked for a check-up within one month. For moderate hypertension during 1968–69 a saluretic diuretic with potassium supplements was used in the first place if the serum uric acid level was normal Alternatively α-methyldopa and bethanidine possibly combined with hydralazine were used As from 1970 a saluretic diuretic or a β-blocker was used as first-line treatment. If control of the BP was not adequate hydralazine was added to the basic drug In certain severe cases more complicated combinations were used The objective of treatment for patients below 55 years was a BP ≤150/100 mmHg and for older patients a diastolic BP ≤100 mmHg.

Hyperlipidemia Cholesterol ≥300 mg/100 ml and/or triglycerides ≥175 mg/100 ml were treated by dietary measures In certain cases, and after special clinical assessment clofibrate and/or nicotinic acid were also given

Smoking At the time of the first contact, while the patient was receiving acute care oral and written anti-smoking counselling was given The importance of strict abstinence from smoking was also emphasized during the follow-up *A sedative* was given if necessary to help the patient stop smoking.

Anticoagulants Patients with enlargement of the heart and concurrent atrial fibrillation (either stationary or recurrent) and/or left ventricular aneurysm and/or signs of extensive arteriosclerotic disease for example intermittent claudication prior to infarction were given long-term treatment with dicoumarol

As regards other diseases and symptoms the principle was to care for the patient at the Cl nic to the greatest possible extent either directly or indirectly via referral to a suitable specialist The management of patients on prolonged sick-lea e was discussed at regular meetings between the physicians and nurses from the Postmyocardial Infarc

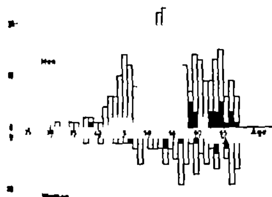


Fig 1 Patients cared for at the Postmyocardial Infarction Clinic during 1968–70 by age and sex. ■=patients who came under care due to reinfarction N B 56-year-old comprise only patients who had their infarct during 1968–70, 57–67-year-olds comprise only patients from 1970

tion Clinic the physicians from the Rehabilitation Clinic and the medical advisors and staff of the Göteborg Regional Social Insurance Office

PARTICIPATION RATE AND SUMMARY OF THE NATURAL HISTORY

During 1968–70 479 patients (391 men, 88 women) were cared for at the Postmyocardial Infarction Clinic These patients included 64 men and 16 women who had previously suffered an MI and who thus came under the care of the Clinic due to reinfarction (Fig. 1) Few patients failed to accept the *offer of controls at the Clinic* At the first visit four of the 479 patients did not attend. The cumulative number of absentees up to and including 2 years follow-up was 13 of 414 survivors i.e 3%

One year afte infarction 56% of the survivors were back at work, 26% were on sick-leave and 18% were receiving disability pensions For those who had returned to work the median duration of sick-leave was 19 weeks At the control 1 year after infarction 47% of the patients were receiving digitalis and 19% were taking a diuretic.

Certain aspects of the infarction studies which began in 1968 have been published The characteristics of young female infarction patients were presented n a thesis in 1973 (3) In the same year studies on exercise tolerance and ability to undergo physical training after MI were presented in another thesis (24) The characteristics of infarction patients compared to the general population (9) the natural history during the acute phase and during follow-up (38) and prognostic aspects (30) have been presented in three other theses

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CREATINE PHOSPHOKINASE AFTER SUBMAXIMAL PHYSICAL EXERCISE IN UNTRAINED INDIVIDUALS

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Abstract Serial estimations of total serum creatine phosphokinase (CPK) have been performed before and during 18–49 hours after submaximal physical exercise in 17 untrained individuals, mean age 50 years. The maximal CPK increase after exercise was 32 mU/ml (73%). The serum CPK did not exceed the upper normal limit (130 mU/ml) except in one individual (150 mU/ml). The maximal CPK increase in patients with acute myocardial infarction (AMI) varied between 101 mU/ml (133%) and 2 260 mU/ml (3 790%), mean 900 mU/ml (1 184%). As the maximal CPK elevation in AMI occurs within the same period it seems that heavy physical work of short duration just before the onset of symptoms will very seldom impair the diagnosis of AMI with the CPK technique used

Serial estimations of serum creatine phosphokinase (CPK) have gained much importance in the diagnosis of acute myocardial infarction (AMI) As CPK rises earlier above normal level and reaches its maximum earlier than other commonly used enzymes it provides a very valuable test in the early phase of AMI (3 11 15 19) CPK has been documented to have a very high sensitivity in AMI and the CPK range is about double the GOT range in the same patients However there has been much argument about the specificity Significant CPK elevations have been reported after e g. physical exercise (1 9 11 14, 18) i m injections of various drugs (?) cardioversion of arrhythmias (6) and in muscular diseases (5) On the other hand, these studies have been performed with rather infrequent blood sampling mostly only once a day

The aim of the present study was to investigate the change in total CPK after submaximal physical exercise in untrained individuals of different ages by frequent blood sampling.

MATERIAL AND METHODS

Seventeen untrained subjects, 15 men and women, performed a stepwise increasing exercise on a bicycle ergometer Their ages ranged from 32 to 73 years (mean 50). The starting load for the men was 50 W with increments of 50 W every 6 min The women started on 40 W with increments of 30 W every 6 min as far as possible None had any history of angina pectoris or myocardial infarction and none experienced chest pain during their exercise test. No i.m. injections were given before or after the test

Venous blood samples for total serum CPK analysis were taken 30 min before, just prior to the bicycle test, immediately after 30 min after 1 hour after and every 2nd–4th hour for 18–49 hours. The blood sample was centrifuged and the serum was frozen to −18°C for 1–3 days. All blood samples from one individual were analysed together using the same CPK reagent for the whole batch The CPK activity was measured at 37°C with spectrophotometer (LKB Reaction Rate Analyzer 8600) using a test pack (Boehringer® 15771). The CPK activity is expressed in mU/ml (Enzyme nomenclature Recommendations 1964 of the International Union of Biochemistry Elsevier Amsterdam, London and New York 1965).

The maximal difference when analysing double samples varied from 0 to 56 mU/ml for CPK values between 16 and 2 186 mU/ml Comparison of the CPK activity in samples analysed immediately and after freezing for 1–41 days (mean 8) showed a difference varying from 0 to 42 mU/ml, for CPK values between 21 and 1 125 mU/ml These differences are not statistically significant ($p > 0.05$).

RESULTS

The 17 individuals stopped their exercise test on 40–300 W (mean 130±67) because of muscular fatigue None experienced any chest pain. The CPK values are shown in Fig. 1 The maximal CPK elevation immediately after the exercise test was 1

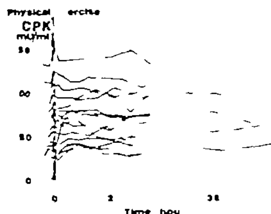


Fig 1 CPK following physical e ercise (indicated by arrow)

mU/ml (18%) The maximal CPK increase during the following 18–49-hour period was 3 mU/ml (73%) *In no subject did CPK rise above 150 mU/ml* after exercise and only one individual had a CPK value above the upper normal level (130 mU/ml with our technique)

In 97 consecuti e AMI patients the maximal CPK increase varied between 101 (133%) and 760 mU/ml (3 790%) mean 900 mU/ml (1 184%)

DISCUSSION

e effect on serum CPK activity of various forms ysical exer se ha been variously reported by rent authors (1 8–14 16 18 20) The conflict results may be due to differences in age and physical state of the subjects the nature and duration of the exercise the ntervals of blood sampling the duration of the follow-up after exercise and different methods for CPK analysis

Most of the studies mentioned concern young healthy students trained or untrained and young athletes In the present study the mean age (50 years) was not as far from that of patients with AMI and only minimal CPK ele ations were observed in each individual Minimal CPK elevations are uncommon in AMI diagnosed by history ECG and serum GOT/OPT Hea y physical exercise just before the onset of symptoms is also uncommon So diagnosing AMI with the aid of serial CPK determinations will very seldom be impaired by heavy physical work of short duration prior to the admission.

Assuming that the observed CPK elevations after exercise are due to the skeletal muscle release it should be possible for diagnostic purposes to differentiate this source from the myocardium by using the CPK isoenzymes. There are however divergent opinions about the proportion of CPK MB in skelet l muscle 0–20% (4 17 19) as well as about the proportion of CPK MB as a percentage of total CPK in association with AMI 0–38% (7 19). There is accordingly a certain risk of a false positive as well as a false negative diagnosis of AMI even with the aid of CPK MB isoenzymes

In conclusion only insignificant elevations of total CPK occurred during 18–49 hours after submaximal physical exercise Heavy exercise performed within this period will therefore apparently only seldom interfere with the diagnosis of AMI by serial estimation of total CPK

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GAUCHER S DISEASE AND BENIGN MONOCLONAL GAMMAPATHY

A Case Report with Immunofl oresc nc Study of Bone Marrow and Spleen

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Abstract A case of chronic non-neuronopathic Gaucher disease is presented The diagnosi w based on the finding of typical Gaucher cells in the bone marrow and spleen. The patient also had a benign monoclonal gammapathy Immunofluorescence studies of bone marrow and spleen were performed using monospecific antisera against immunoglobulin heavy and light chains. The results indicate a monoclonal plasma cell proliferation in the bone marrow whereas the distribution of plasma cells in the spleen was polyclonal. The combination of chronic Gaucher's disease and monoclonal gammapathy wa also found in the patient brother

The lipid storage disease first described by Gaucher in 1882 (6) is characterized by accumulation of glucosyl ceramide in reticuloendothelial cells of various tissues particularly the liver pleen and bone marrow Two clinical forms can be distinguished neuronopathic Gaucher s disease which can be acute or subacute and is seen in infants and young children, and chronic non-neuronopathic Gaucher's disease afflicting older children nd adults (4) The chronic form has a much more protracted course and clinical manifestations may be absent until late adult life. It is also much more common (10-70 times) than the infantile type (1) Chronic Gaucher s disease is generally seen in Ashkenazy Jews It is a hereditary condition and the autosomal recessive nature of the disease is well established (5)

The biochemical basis of the disease is deficient activity of the enzyme glucocerebrosidase (?) This leads to ineffective breakdown of various glycolipids and excessive accumulation of glucosyl ceramide in reticuloendothelial cells. They tak on a characteristic appearance with a wrinkled or striated cytoplasm and have been named Ga cher cells The most common clinical ympton are due to accumulation of such cells n the liver and pleen leading to hepato- and splenomegaly and linical signs of hypersplenism (4). Bone marrow n ol e ment is common and typical roentgenographic bone lesions are seen in 50-75% of the cases (4) Other common symptoms are pinguecula and ellow brown skin pigmentation A high level of non tartrate-inhibitable serum acid phosphatases is helpful in making the diagnosis (17), which s confirmed by the presence of Gaucher cells in bone marrow aspirates. An interesting aspect of chronic Gaucher s disease is the occurrence of serum immunoglobulin abnormalities These include both polyclonal immunoglobulin increase and monoclonal gammapathy (7 13 15 16 19 21)

We report a case of Gaucher s disease with a monoclonal gammapathy without clinical signs of *multiple myeloma*. By immunofluorescence studies the M-component producing cell clone was traced to the bone marrow but ot to the spleen

CASE REPORT

The patient is 55-year-old Ashkenazy Jewish woman who wa referred to the Department of Medicine in 1968 because of fatigue and arthralgia. Her past history included operation for inguinal hernia, appendectomy and cholecystectomy The physical xamination revealed nothing abnormal. There were no pinguecula, no abnormal pigmentation and no hepato- or splenomegaly She had moderate normochromic anemia and slight leucopenia and thrombocytopenia. Hb level was 10 1 g/100 ml RBC ..9 mill./mm WBC 3 100 mm³ and platelets 110000/mm³ ESR was 37 mm/h Plasma protein analysis using agarose gel electrophoresis, immunoelectrophoresis and

monoclonal gammapathy splenic tissue was ex amined post mortem The distribution of immunoglobulin-containing cells was polyclonal The bone marrow was however not investigated

An alternative explanation is a common genetic predisposition to Gaucher s disease and immunoglobulin disturbances. Chronic Gaucher s disease is known to be inherited as an autosomal recessive trait (5) It would be interesting to study the incidence of polyclonal and monoclonal hypergammaglobulinemia in relatives of patients with this disease As far as we know no such study has been performed The coexistence of Gaucher s disease and an M-component of the same immunoglobulin class in our patient s brother is noteworthy Further characterization of the M-components including use of anti-idiotypic antisera is intended

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